MEASUREMENTS OF THE UTILIZATION OF MAGNESIUM FROM DIFFERENT OXIDES IN RUMINANTS

BY

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MEASUREMENTS OF THE UTILIZATION OF MAGNESIUM FROM DIFFERENT OXIDES IN RUMINANTS

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Five experiments were conducted to investigate the use of urinary magnesium excretion immediately following high level supplementation as a bioassay criterion to determine the relative Mg availability of different Mg oxides in sheep. In Experiment 1 sheep were fed a semipurified diet supplemented with 600 mg/kg Mg as sulfate or four feed grade Mg oxides varying in origin and particle size. Urinary and fecal collections were made during the experimental period and results obtained from the balance data were compared with results obtained from Experiment 2. In Experiment 2 sheep were fed a natural corn-soybean meal-cottonseed hulls diet supplemented with 0, 700, 1400 or 2100 mg/kg Mg as sulfate or 1400 mg/kg Mg as three of the oxides used in Experiment 1. Results of Experiments 1 and 2 indicated that Mg absorption was the most reliable criterion to compare Mg availabilities. In Experiment 2 good correlations between Mg absorption and urinary Mg excretion were obtained. Best results

were obtained when urinary excretions for days 4 and 5 were used. In Experiment 3 the same basal diet as in Experiment 2 was used. Dietary treatments included the basal diet supplemented with 1000, 2000 and 3000 mg/kg Mg as sulfate or 1000 and 3000 mg/kg Mg as raw magnesite. Urinary Mg excretions and balance data indicated poor utilization of Mg when supplemented as raw magnesite. When different methods were used to calculate the percent availability of Mg in raw magnesite, results indicated that relative values for availability were subject to the method of calculation. Experiments 4 and 5 were 10-day balance trials conducted to determine Mg availability from different feed grade Mg oxides. In Experiment 4 the relative availabilities of three oxides were determined. Using reagent grade as the standard reference source, dietary supplementation included 0, 700, 1400 or 2100 mg/kg Mg as sulfate. The feed grade oxides were supplemented at 1400 mg/kg. Balance data and urinary Mg excretions for days 4 and 5 were used to measure Mg availability. For Experiment 5 the same procedure as Experiment 4 was used to compare relative Mg availabilities of two feed grade oxides and one feed grade oxide, which was also used in Experiment 4. Analysis of the data indicated that the relative availabilities obtained with balance data were consistent with those obtained with the use of urinary excretion.

CHAPTER I
INTRODUCTION

Magnesium (Mg) is the eleventh most abundant element on earth. About 2 percent Mg is present in the earth's crust and about .135 percent in seawater. Magnesium occurs widely in nature as carbonates, (dolomite and magnesite); silicates, (olivine, serpintine, talc and asbestos); as sulfates, (Epsom Salts ($MgSO_4$)) and chlorides (MgCl2) in seawater, brine wells and salt deposits.

Magnesium has been accepted as an essential nutrient for the rat since 1933 when Kruse et al. (1933) induced an acute Mg deficiency syndrome in rats. Soon thereafter there was a flurry of interest in the possibility that Mg also might play a role in man and farm animals. Haury (1942) correlated low plasma levels of Mg in man with several clinical conditions in which hyperirritability or convulsions were present. Evidence for Mg deficiency in livestock was first provided by Sjollema (1929) who found that grass staggers of cattle and the convulsions of calves fed only whole milk were similarly found to be associated with low blood Mg levels, and to be preventable by feeding Mg supplements. Autopsy examination of the cows and calves that died in acute Mg deficiency revealed severe cardiovascular damage, including necrosis and calcification.

In the past fifteen to twenty years, the early work on the

Mg deficiency syndrome has been verified and interrelationships of Mg with other dietary factors have been eludicated.

The systems affected in an acute deficiency state are primarily neuromuscular, cardiovascular, and renal. Hyperirritability that terminates in convulsions, hyperemia, vasodilation and trophic changes in the skin are initial manifestations of an acute Mg deficiency. Calcinosis of heart, muscle and kidney occur in a later stage.

Hypomagnesemia continues to be a serious disease of grazing livestock and is responsible for considerable economic losses. Chronic hypomagnesemia arises from a prolonged deficiency while acute hypomagnesemia occurs as a "short term" deficiency induced by a complex interaction of plant and animal factors. Since the body has no homeostatic mechanism for controlling the level of Mg in the extracellular fluid, the maintenance of normal levels depends on adequate and continuous absorption of Mg from the gut. Anything which affects this supply will predispose the animal to hypomagnesemia and, despite the many prophylactic measures that have been recommended, the most effective preventive measure remains dietary Mg supplementation. Because the effectiveness of Mg supplements depends on the utilization of the element by the animal, careful consideration of biological availability of supplements is required. Several methods and criteria have been used to determine the bioavailability of Mg sources and each method has its own advantages and disadvantages (Thompson, 1965; Reid, 1983). Bioassays are complicated by the fact that Mg does not have a radioisotope with a sufficiently long half life to

permit routine estimations of endogenous Mg excretions which are necessary to calculate "true availability". Semipurified diets, which are expensive and often unpalatable, have been used in determinations of Mg availability at low intakes. However, these diets are impractical and have been used with limited success.

Recent experimental evidence has shown that a high correlation exists between urinary Mg excretion and Mg intake in excess of the requirement (Chicco et al., 1972; Bouwman, 1978). This makes urinary Mg excretion a valuable response variable to use in bioavailability studies. A rapid, sensitive and inexpensive method using urinary excretion immediately following high level supplementation may be useful as an indicator of Mg bioavailability.

This study was undertaken to compare different methods of determining relative bioavailability of several feed grade Mg salts using reagent grade Mg sulfate as a standard reference source.

CHAPTER II
REVIEW OF LITERATURE

Several excellent books and reviews concerning the physiological roles of Mg, clinical aspects, pharmacological properties and other phenomena have been published (Simesen, 1970; Aikawa, 1963; Littledike and Cox, 1978; and Fontenot et al., 1983). These publications, some of which are proceedings of workshops and symposia, bring together a wealth of information on several aspects of Mg in man, animals and plants. The objective of this review is to present selected phenomena that hold promise for the development of concepts for a better understanding of Mg utilization by animals and factors influencing the utilization of dietary Mg supply.

Magnesium Metabolism

Magnesium is the fourth most abundant cation in the body and the second most plentiful intracellularly (Wacker and Parisi, 1968). The Mg content of most adult animals is about .05%, but is substantially lower in newborns. Magnesium is closely associated with calcium and phosphorus in its distribution and metabolism. About 50 to 70 percent (Rook and Storry, 1962; Todd, 1969) of total body Mg is found in bone. Of this supply, about one third is subject to mobilization for use in soft tissues when intake is inadequate. The remainder of Mg in the body is equally distributed between muscle and tissue. In animal physiology

magnesium fulfills many functions that hinge on two main properties: (1) enzyme activation, and (2) competition with calcium in the exitation secretion coupling process. Although the majority of Mg in the body is in the skeleton, its major function is located inside cells. In the cell Mg is actively involved in biological processes such as synthesis of ATP, membrane transport, synthesis of fat, protein and nucleic acids and oxidative phosphorylation. Disturbances in Mg metabolism will affect important physiological properties such as growth, immunity, muscular contraction, red blood cell survival, lipid metabolism, sodium and potassium metabolism, metabolism of collagen rich tissues, calcification of the kidney and occurrence of neoplasms.

Absorption

Previously, it was assumed that the small intestine was the major site of Mg absorption (Phillipson and Storry, 1965). However, more recently, it has been shown that the main site of Mg absorption in ruminants is localized in the forestomach rather than in the intestine as known for monogastric animals and pre-ruminating calves and lambs (Grace and MacRae, 1972; Grace et al., 1974; Thomas and Potter, 1976a, 1976b; Greene et al., 1983a, 1973b). Rogers and van 't Klooster (1969) demonstrated that the absorption of Mg from the forestomach was essential for maintaining the physiological blood Mg concentrations. In their experiments animals were used where the forestomach was bypassed with adequate amounts of Mg. This resulted in hypomagnesemia. Although most experiments done with ruminants have indicated that

Mg absorption occurs in the forestomach. There is some uncertainty about the localization of Mg absorption within this area. Grace and MacRae (1972) and Kemp et al. (1973) have reported changes in the relative importance of the forestomach region as a site of Mg absorption due to level and method of feeding. Recent experiments with calves have implied that the omasum may be the main absorption site (Horn and Smith, 1978), but Martins (1985) also demonstrated significant absorption of Mg from the rumen of calves using the washed reticulo-rumen method. To investigate this phenomenon more closely, Gabel and Martins (1985) conducted experiments on heifers that were fistulated in the dorsal rumen sac. Solutions containing different concentrations of $MgCl_2$ and CR-EDTA were infused and mixed with ruminal contents. Observations were then made for the ratios of Mg: Cr in the ruminal fluid. In all the experiments, the Mg concentrations in the ruminal fluid fell faster than the Cr concentrations indicating either an outflow to the omasum or an increase in Mg absorption in the rumen. Results from this experiment also indicated that at higher Mg concentrations in ruminal fluid, absorption of Mg was decreased. Gabel and Martins (1985) suggested that the contradictions between their findings and those of Horn and Smith (1978) were due to the differences in Mg concentrations used in the ruminal fluid. Horn and Smith (1978) used concentrations of 12-15 mmol/l. At these concentrations it was likely that the transport of Mg had been saturated and, as a result, the amount of Mg absorbed over time was very small compared with the large pool. In another

experiment Rahnema and Fontenot (1983) studied the effect of Mg supplemented from MgO and dolomitic limestone on digestion and absorption in sheep. They found that the major site of Mg absorption was the preintestinal region irrespective of the Mg source used. Magnesium supplementation resulted in an increase in preintestinal Mg absorption with Mg oxide having the highest value. It appeared that the greater utilization of Mg from the oxide was due to a greater absorption of Mg from the forestomach region. Greene et al. (1983b) also investigated the site of Mg absorption and concluded that the main site of Mg absorption occurred in the preintestinal region.

Although several studies have shown that in addition to occuring in the rumen, the absorption of Mg can also occur at sites distal to the pylorus (Grace and MacRae, 1972; Field and Munro, 1977) it is not clear whether Mg absorption from both of these sites can vary in proportion to Mg supplementation. An experiment undertaken by McLean et al. (1984) to study this phenomenon showed that the bulk of Mg absorption occurred before the pylorus during the control and first infusion periods. The net Mg absorption proximal to the pylorus rose with declining efficiency as Mg intake was increased.

Transport

In vitro studies using sheep ruminal epithelium indicated the existence of an active transport across isolated ruminal epithelium. Martins et al. (1978) demonstrated that the transport of Mg across the ruminal epithelium could be inhibited by using quabain, a known Na^+/K ATPase inhibitor. In these

experiments the transport of Mg exhibited several characteristics that are characteristic of an active process, i.e. saturation, temperature sensitivity, and competitive inhibition. In another experiment Martins (1985) studied the effect of dinitrophenol, another ATPase inhibitor, on Mg transport across an isolated preparation of sheep ruminal epithelium. Under normal physiological conditions a significant net effect of Mg was observed, in which efflux was 7-8 times that of the influx. But in the experiments, in which ATP synthesis was blocked by addition of dinitrophenol, the flux of Mg from the mucosal to the serosal side was significantly reduced. These observations are consistent with the conclusion that Mg absorption across the ruminal wall is an active process.

The mechanism of Mg absorption is a model that is conceived as four pools connected in series. Magnesium from the diet enters the omasum as a solid phase. The Mg particles in the solid phase can then move from the interior of the solid phase to the surface. Once on the surface these Mg particles together with the saliva can enter the liquid phase which is considered the main pool for plasma Mg (Field, 1983). Studies on the mechanism of Mg absorption (Martins et al., 1978; Field et al., 1977) have suggested that there are two main factors that control the absorption of Mg: 1) the concentration of Mg in the liquid phase and 2) changes in the efficiency of Mg transport through the gut wall. Evidence for these conclusions in part come from in vitro studies by Martins et al. (1978) with isolated ruminal mucosal preparations. They observed a curvelinear relationship between

Mg absorption and Mg concentration at the mucosal side of the ruminal epithelium, which indicated that the Mg absorption became less sensitive at the higher concentrations. Similar observations were made by Field and Munro (1977) who studied the extent of Mg absorption into the gastro intestinal tract of sheep. The absorption of Mg appeared to be less sensitive at the higher Mg concentration in the ruminal liquid. This means then that any dietary factor that is capable of altering the concentration of Mg in the liquid phase can also influence Mg absorption, particularly at low concentrations.

The concentration of Mg in the liquid phase of the digesta, depends a great deal upon the solubility of dietary Mg. Because Mg has to be in an ionized form to be absorbed, it is likely that the solubility of Mg is closely related to Mg bioavailability. Ammerman et al. (1972) tested the solubility of Mg from different Mg salts and found correlations between solubility in hydrochloric acid and Mg absorption. Bouwman (1978) also reported good correlations between relative Mg absorption and solubility in .4% HCl and 2% citric acid. Smith and Horn (1976) found that the solubility of Mg released from feed is a function of pH in the ruminal fluid. They reported that a change in pH from 5.5 to 7 was associated with a 50% reduction in the soluble fraction. Most of the effects, however, were observed when pH range was between 6.5 and 7.

Although the existence of a negative relationship between ruminal pH and Mg absorption is in no doubt, the explanation of increased absorption due to changes in Mg solubility has its

critics. Horn and Smith (1978) reported that changes in Mg absorption can occur over pH ranges which would be without effect on Mg solubility. These effects can be expected when changes in ruminal pH are induced by such diverse feedstuffs as herbage and cereals. Another explanation for this phenomenon given by Martins and Rayssiquier (1979) is that the VFA concentration which is responsible for the ruminal pH acts directly on the transport mechanism by increasing blood flow through the ruminal wall (Martins and Raysiquier, 1979). As a result an increase in VFA concentration will cause an increase in Mg absorption.

Another dietary factor that is known to affect the transport mechanism across the gut wall is K. Greene et al. (1983a, 1983b) found that increasing levels of dietary K resulted in a linear increase in fecal Mg and a linear decrease in Mg absorption. Lambs fed the high level of Mg (.2%) absorbed and retained more total Mg compared with the low Mg level (.1%). In a similar experiment with steers Greene et al. (1983a) found that preintestinal Mg absorption was decreased by 39% at the highest level of K supplementation (4.8% dry basis) and serum Mg decreased with increasing levels of K supplementation. It is not known how a high intake of K inhibits Mg absorbtion, exept that it probably acts through the transport mechanism. Scott (1966) showed that a high K intake reduces the net absorbtion of Mg by increasing the back flow of Mg ions from blood to rumen content. Newton et al. (1972) used ^{28}Mg to show that increased fecal Mg excretion resulted from a decrease in Mg absorption rather than from an endogenous loss of Mg.

Most test diets used to evaluate the influence of K upon Mg absorption have contained constant Na and variable K thereby altering the dietary K:Na ratio. To investigate the effect of both Na and K on Mg availability, Poe et al. (1985) conducted two metabolism trials with altering dietary Na and K concentrations. Increased K and Na intakes resulted in increased fecal Mg excretion in lambs. Both elevated K and K+Na concentrations depressed urinary Mg excretion. Addition of Na to a diet high in K did not enhance Mg absorption in lambs. It is not known how K inhibits Mg absorption; however, it is speculated that the effect is on the transport mechanism (Martins and Rayssiquier, 1979).

An organic dietary constituent which is thought to decrease the solubility of Mg in the liquid phase of the gut is lipid. Kemp et al. (1966) showed that fecal Mg excretion of dairy cows was increased when lipids were added to the diet. Magnesium in the feces was excreted as Mg-soaps and lower plasma Mg levels were observed. However, in another study, Scott (1966) did not observe soap formation in the stomach region when corn oil was infused into the rumen of sheep. He concluded that the pH of the rumen was not favorable for the formation of fatty acid soaps. These results cast doubt on hypothesis that soap formation is the main mechanism by which Mg absorption is decreased.

The Mg concentration in the ruminal fluid is largely dependent upon the rate of water movement and therefore water intake will also affect absorption of Mg. The transport of Mg through intestinal epithelium occurs in two ways, transcellular and paracellular. The transcellular transport is active whereas

the paracellular passage is passive. As a result the Mg dissolved in water can pass paracellular and passive as a result of solvent drag. In experiments with sheep Laue (1980) observed paracellular Mg transport in the colon that was a result of solvent drag. Stout et al. (1967) observed enhanced Mg absorption at high water intakes. Using the isolated reticulo-rumen of sheep, Martins (1985b) studied the effect of water absorption on Mg absorption in the rumen. Results obtained from this experiment showed that the altered osmotic pressure of the buffer solutions led to substantial changes in the net transport of water through the rumen wall, but the net Mg efflux was not affected by water movement. Martins (1985b) pointed out that the epithelial structure of the colon and the tight cell junctions characteristic of the rumen wall are the major reasons why Mg transport remained unaffected by the movement of water.

Large amounts of dietary Ca are known to aggravate the severity of an Mg deficiency and increase the Mg requirements. Chicco et al. (1973a) reported that high dietary Ca depressed the Mg content in bone and plasma and decreased Mg utilization. Conversely a high Mg diet reduced plasma Ca. Reed et al. (1974) reported that supplementation of cattle with bonemeal increased inorganic P levels but depressed Ca and Mg concentrations in serum. High protein diets were also found to be the cause of increased Mg requirements (Colby and Frye, 1951). Fontenot et al. (1960) observed that a diet containing 33.3 percent protein and 1.35 percent K increased fecal Mg and decreased plasma Mg. Stillings et al. (1964) reported that an increase of nitrogen

content of forage from 2.11-2.62 percent to 3.74-4.38 percent decreased Mg utilization.

On the effect of dietary factors on Mg absorption, attention also has focussed on the effect of high ammonium levels in ruminal fluid on the absorption of Mg. Grass staggers, a conditioned Mg deficiency, can be reproduced by feeding a normal diet supplemented with ammonium carbonate in amounts sufficient to raise the ammonium content of the rumen to that encountered in the spontaneous disease (Head and Rook 1957). In a similar experiment with lambs (Henry et al., 1977) the high ammonium content induced reduction in plasma Mg and signs of tetany appeared in 50 percent of the lambs given the ammonium salts. Other researchers (Fontenot et al., 1973 and Moore et al., 1972), however, failed to reproduce these effects with ammonium fertilization on pasture. Failure to produce the effects was attributed to some alteration in the plant other then an increase in nitrogen content. An increase in K content has been suggested. Martins and Rayssiquer (1979) concluded that very high levels of ammonium are required to produce a significant decrease in Mg absorption. These high concentrations of ammonia in the ruminal fluid will only be caused by a high ammonium production and low microbial activity ,e.g., diets high in soluble nitrogen and low in energy.

Excretion

Excretion of Mg in ruminants is by both fecal and urinary routes. Fecal Mg consists of the unabsorbed portion of the Mg consumed and Mg excreted into the gastrointestinal tract from

bile, saliva and other digestive secretions. It is believed that some of the secreted Mg is reabsorbed but little is yet known about the conditions that control endogenous Mg excretion. Rook and Storry (1962) have suggested that endogenous excretions of Mg are likely to be increased by the greater flow of saliva stimulated by bulky diets such as roughages and grass. Simesen et al. (1962) measured endogenous Mg excretions of 3.5 and 1.5 mg/kg body weight for calves and cows, respectively. Using regressions of Mg intake and total Mg excretions (fecal and urinary), Bouwman (1978) estimated an endogenous fecal Mg excretion of 2.25 mg/kg of body weight in sheep. This result agreed with other results with sheep reported by Chicco et al. (1972) and Meyer et al. (1973), who calculated endogenous Mg excretions of 2.3 and 2.6 mg/kg of body weight, respectively.

Kidney function is also important in Mg homeostasis. Renal handling of Mg is primarily a filtration reabsorption process in which a fraction of the filtered Mg is reabsorbed and the remainder is excreted in the urine. Urinary Mg is elevated when the filtered load exceeds the maximal tubular reabsorption capacity. However, urinary Mg conservation can be mediated by the parathyroid hormone, thereby controlling the plasma Mg levels. The relationship between urinary Mg excretion and plasma concentration is exponential (Kemp et al., 1961; Meyer et al., 1973, and Bouwman, 1978) which demonstrates the ability of the kidney to decrease the Mg excretion during hypomagnesemic conditions. Estimates of the renal threshold values of plasma Mg concentrations range from 1.4 to 2.0 mg per 100 ml (Rook et al.,

1958; Storry and Rook, 1963; Chicco et al., 1972, and Bouwman, 1978). Urinary excretion of Mg is influenced by the concentration of Mg in the plasma above the renal threshold, which is about 1.8 mg per 100 ml. If the plasma level is above the renal threshold, the relationship between Mg absorbed and Mg excreted in urine is linear (Kemp et al., 1961; Rook and Campling, 1962; Storry and Rook, 1963; Ammerman et al., 1972; Chicco et al., 1972; Bouwman, 1978).

Milk can also be an important route of Mg excretion. In 1978 the Agricultural Research Council predicted a mean value of 126 mg Mg per kg milk. Storry and Rook (1962) quote a range of published values from 70 to 180 mg per kg of milk, and provide evidence that no significant decline in Mg content of milk occurs when intake of Mg is reduced.

Magnesium Requirements

The Mg status of cattle is best assessed from Mg concentrations in blood and in urine (Committee on Mineral Nutrition, 1973). Serum Mg concentrations of 1.0 to 2.0 mg per 100 ml are considered deficient and a concentration less than 1.0 mg per 100 ml indicates danger of tetany. Underwood (1966) considered that 2.0 to 3.2 mg Mg per 100 ml serum is the normal range for cattle. Kemp (1960) observed a positive correlation between serum Mg levels and Mg content in forage and found that when herbage contained more then .2% Mg (dry basis), serum levels in experimental animals were normal. O'Kelly and Fontenot (1973) calculated that gestating beef cows required 8.5, 7.0 and 9.0 g of dietary Mg per day for 175, 200 and 255 days of gestation,

respectively, to maintain a serum Mg concentration of 2.0 mg per 100 ml. On the basis of 6.8 kg daily feed intake, dietary Mg concentration was calculated to be 1250, 1030 and 1320 mg/kg for these three periods. The same authors determined the Mg requirements of mature lactating Angus cows at 35, 95 and 155 days post partum. Based upon regression equations for dietary Mg and serum Mg they estimated the requirements to be approximately 1800, 1900 and 1600 mg/kg for the three respective stages of lactation. In metabolism trials with lactating cows, Kemp et al. (1961) calculated the minimum daily requirement to be about 2.5 g for maintenance plus .12 g Mg for each kg of milk. These values, however, were low compared to the ones of Blaxter and McGill (1956), who calculated a minimum Mg requirement of 5.4 g daily per cow and .48 g Mg for each kg of milk. The NRC (1984) recommended 1800 mg/kg Mg on a dry basis for lactating beef cows and 2000 mg/kg for lactating dairy cows.

Blaxter and Rook (1954) reported that calves which died from hypomagnesemic convulsions showed Mg concentrations in rib bone ranging between .19 and .35 percent of bone ash. Concentrations of normal calves ranged between .67 and .70 percent. Smith (1959) fed calves with low Mg milk and observed that Mg concentrations in bone ash fell progressively from a normal value of .75 percent to about one third of the value when serious clinical signs appeared. Taylor and Winter (1929), using lactic acid extractions on bones of calves and oxen found that a greater percentage of original Mg was removed from calf bones then from ox bones. This proved that calves are able to mobilize Mg

reserves in bones relatively more easily then mature cows. Huffman et al. (1941) calculated a total daily intake of 30 to 40 mg/kg body weight to maintain normal plasma levels in calves fed Mg salts. Blaxter and Rook (1954) calculated the requirement of 30 to 35 kg Ayrshire bull calves to be 16.2 mg/kg of body weight. The NRC (1986) lists requirements ranging from 400 to 1000 mg/kg Mg for growing beef calves while the level of a growing calf of a dairy breed was set at 1600 mg/kg.

Using total dietary intake as criterion, Ammerman et al. (1970) found that the minimum amount of Mg (as oxide) required by sheep 18 months of age was between 8 and 10 mg per kg of body weight or a dietary concentration of 333 mg/kg. Chicco et al. (1972), who used regressions to determine the daily requirement of sheep, estimated a requirement of 4.03 mg Mg per kg body weight. Field et al. (1958), who used the relationship between Mg absorption and urinary Mg excretion, estimated that the Mg requirement for maintenance in sheep ranged from 800-950 mg Mg per head daily. Requirements listed by the NRC (1985) range from 400 to 800 mg/kg for all classes of sheep.

Bioavailability of Magnesium

Techniques for Its Measurement

While there is general agreement on the significance of the availability of Mg, it is still unclear how Mg availability should be expressed. Many criteria have been used to compare availabilities of Mg, e.g., percent utilization, apparent availability, apparent absorption, true absorption, absorption, net retention, and true availability. Estimation of true

absorption depends on the accuracy of measuring metabolic fecal Mg. In practice endogenous Mg is difficult to measure and therefore values for true absorption are scarce in the literature. Despite these difficulties several authors have tried to estimate endogenous Mg excretions. Care (1960) and Simesen et al. (1962) used a radioisotope technique to evaluate the metabolic Mg in sheep and cattle. Ammerman et al. (1972) calculated the true absorption by correcting intake and excretion of the supplemented diets with the intake and excretion results obtained in non-supplemented diets. However, as Thompson (1965) pointed out, this method is unreliable since absorption from the non-supplemented diet may be enhanced by depletion of Mg from the body thereby affecting the results on true absorbtion.

More frequently used in ruminant studies are measurements of the difference between intake and fecal excretion expressed as apparent absorption (also apparent digestibility or apparent availability). While the apparent absorption is the least satisfactory expression, the simplicity of the method, which requires only a knowledge of intake and total fecal excretion, has comment itself to many workers. Most of the information on Mg utilization by ruminants has been calculated on this basis (Rook and Campling, 1962). However, these authors stated that in cases when the apparent absorption of dietary Mg was low the errors of the balance study became large and are not valid for comparisons. The inclusion of urinary as well as fecal excretions allows the calculation of "net retention". The important difference between net retention and apparent

absorption is that the latter represents the amount of the element that has been absorbed from the intestinal tract, whereas net retention includes the fate of the element within the animal. The hazards of interpretation of net retention in conventional balance trials were well recognized (Duncan, 1966), and Fishwick and Hemingway (1973) discussed the high values for Mg retention reported in literature. Another major disadvantage with using net retention is that all the errors involved in sampling, analyses of the diet, feces and urine are added up in the calculations (Bouwman, 1978). In addition, correlations between net retention and intake will be low because the average net retention in a healthy animal approximates zero. As with apparent absorption, net retention does not take into account endogenous losses, and consequently dietary intakes below the maintenance level result in negative values. Another expression also used to estimate Mg utilization is "percent availability". This expression is a "refinement of net retention by taking into account both fecal and urinary endogenous excretions. Due to the problems involved in balance trials other methods were considered to measure the utilization of Mg in dietary sources. As early as 1929, Sjollema and Seekles (1929) suggested that a relationship existed between low Mg concentrations in the urine and the incidence of hypomagnesemic tetany in cows. Experimental work of Rook and Balch (1958) on Mg metabolism in dairy cows showed that urine is the main excretory route for Mg that is absorbed in excess of the requirement. Kemp et al. (1961) found that subnormal or low serum Mg values occurred only when the

excretion of Mg in the urine was less than 1 g/day. Balance trials with over 60 milking cows in the Netherlands (Kemp et al., 1961) showed that as intake of available Mg increased, urinary Mg excretions also increased. Lower intakes of Mg resulted in a decreased excretion of Mg in the urine and negative retention. Direct relationships between urinary Mg excretion and absorbed Mg were also reported by several other workers (Ammerman et al., 1972; Chicco et al., 1972; and Bouwman, 1978). All these findings provide evidence that urinary Mg excretion is sensitive to dietary Mg intake and therefore justify the use of this criterion as an indicator of available supplemental Mg in the diet.

In other studies with sheep and ruminants, Shockey et al. (1984) used fecal Mg excretions instead of urinary Mg excretions to determine apparent Mg absorption. The basis for this approach was that the regression coefficient (slope) of a plot of fecal Mg vs. Mg intake is an estimate of inabsorbability of Mg from the diet. Experimental results showed that at normal Mg intake, linear and logarithmic equations were both useful in predictions of apparent Mg absorption, while at extreme intakes logarithmic equations appeared to be more useful.

Concentrations of Mg in serum and plasma have also been used as a criterion to express Mg availability. Chicco et al. (1972) reported good correlations between plasma Mg concentrations and dietary Mg supplementation. Amos et al. (1975) used the concentration of Mg in the serum of lactating cows to estimate the effect of dietary and Mg availability from calcite and

dolomite. In a comparative study of diagnostic tests for the assessment of Mg status in cattle, Sutherland (1986) found that in nine of the ten pre-calving dairy herds, the Mg status could be determined with 95% confidence by measurements of serum Mg. However, when lactating herds were sampled, milking was not associated with depressions in serum Mg. Comparing serum Mg with the other sampled parameters, experimental results showed that urinary Mg clearance and creatinine-corrected urinary Mg were more sensitive than serum Mg to Mg supplementation in dairy cows. On the basis of these results it may be concluded that responses in plasma Mg concentrations from dietary Mg levels will depend upon the level and range of Mg supplies. At low levels of dietary Mg the plasma concentration is likely to respond to a small increase of Mg in the diet, but at levels above the requirement, the main part of dietary Mg will be excreted in the urine.

Estimates of feed intake may also provide some indication of Mg availability. Ammerman et al. (1970) observed a 60% reduction in feed intake in sheep after 4 days of feeding Mg-deficient diet. Oral administration of Mg resulted in an immediate response in feed intake. With this technique the researchers were able to produce significant differences in feed intake for the different Mg sources and relative biological availability of the different Mg sources was measured in terms of feed intake response following Mg administration. Studying the relationship between plasma Mg and voluntary feed intake in ruminants, Chicco et al. (1973a, 1973b) found good correlation between these

parameters. Plasma Mg decreased more quickly, and voluntary feed intake was reduced sooner in mature sheep than in younger animals when an Mg-deficient diet was fed.

Retention of Mg frequently has been assessed by measuring the incremental retention in a selected bone. Bone is considered the storage depot for Mg and will reflect Mg supplies over longer periods. This technique can be used only with younger growing animals because the ability to mobilize bone Mg declines rapidly with age (Thompson, 1965). Blaxter and Rook (1954) reported that calves which died from hypomagnesemic convulsions showed lower Mg concentrations in rib bone than normal animals. Smith (1959) fed calves with low Mg milk and observed that Mg concentrations in bone-ash fell progressively from a normal value to about one third of the value when clinical signs appeared. Thomas (1959) demonstrated that calves were able to mobilize Mg reserves relatively more easy than mature cows.

Radioisotopic procedures have also been used to some extent for the determination of Mg availability and true absorption (Field, 1959; Mac Donald and Care, 1959; Care, 1960, and Simesen et al., 1962). Among the difficulties that have hindered research on Mg metabolism is a satisfactory tracer isotope. The radioisotope ^{28}Mg which has been used in experiments has a short half-life of 21.3 hours. Upon injection, ^{28}Mg rapidly disappeared from the circulation and was initially found in soft tissues (Field, 1959) However, over 80% of Mg in the body appeared in slowly exchanging components that have not been accessible to measurement due to the short half-life of ^{28}Mg.

Schwartz (1981) has explored the potential of the stable isotope ^{26}Mg as a biological tracer. From the results obtained he concluded that the role of ^{26}Mg as a tracer was limited. In addition ^{26}Mg has a high natural abundance and can therefore not be used in tracer amounts.

Magnesium Availability

The amount of a mineral element in a particular compound has little meaning unless it is qualified with a correction factor indicating its degree of biological availability for a species of animal. The term biological availability generally indicates the degree to which an element is absorbed and available for use in physiological processes (Peeler, 1972). Several criteria have been used to express the availability of Mg in feedstuffs and natural sources, e.g. percent utilization, apparent absorption, true absorption, net retention, biological availability and others. The relative merits and limitations of each of these criteria have been discussed extensively by Thompson (1965).

More than sixty different Mg-containing compounds are known and the availability of Mg has been shown to vary widely. Early studies carried out by Huffman et al. (1941) compared the availability of different Mg salts on basis of plasma Mg concentrations and found no differences among the oxide, chloride, carbonate and phosphate salts. However, Stewart and Moodie (1956) were able to detect differences between the nitrate salt and the sulfate salt. In their experiments the administration of Mg as nitrate resulted in increases in serum Mg. When Mg was administered as the sulfate salt, increases in

serum Mg were less than increases obtained with nitrate during the same time intervals. Little or no differences in serum Mg values were reported by Thomas (1959) when three Mg salts (carbonate, sulfate, and acetate) were used as supplements to milk for calves. Storry and Rook (1963), who were among the first to use urinary Mg excretions as a measure of biological availability, found availabilities of Mg to range from 14.5 to 49.2 percent for a number of Mg salts. Using Mg-oxide as a reference standard, the supplements had relative values from a low of 54 percent for Mg from silicate up to 158 percent for Mg from citrate. Meyer and Grund (1963), who also measured urinary Mg excretions in two bull calves, found Mg oxide and Mg chloride to give the most favorable results when compared to Mg sulfate and Mg carbonate. Magnesium as carbonate was not absorbed. Using Mg absorption data of beef steers on a low Mg diet, Gerken and Fontenot (1967) determined the availability of Mg in dolomitic limestone to be 28 percent of that of Mg oxide. Ammerman et al. (1972) used measurements of feed intake following Mg depletion, as well as apparent absorption, true absorption, net retention and biological availability to determine the availability of Mg in several salts for sheep. Based upon feed intake data, the relative availability of Mg was 65 percent for magnesium sulfate, 85 percent for reagent grade Mg-oxide and 113 percent for the Mg carbonate. Comparisons were made by using reagent grade Mg sulfate as the standard reference source. Relative availabilities of Mg obtained with balance data were 86 and 113 percent for Mg carbonate and Mg sulfate, respectively.

In studies with Mg phosphate, Hemmingway and Fishwick (1976) observed increases in Mg retention and plasma Mg concentration in growing lambs fed the Mg phosphate diets. Fishwick (1978), who used Mg phosphates derived from seawater, reported increased Mg retention in the supplemented animals when compared to the control group. When Mg retention of sheep fed the Mg phosphates was compared to Mg retention of sheep fed Mg as oxide, no differences were found. When Mg phosphate was given to lactating cows during spring grazing, the normal decline in mean plasma Mg was significantly reduced (Ritchie and Fishwick, 1977).
Young (1973, 1975) studied the availability of Mg in soluble salts and found that, when Mg chloride or Mg sulfate were supplemented in drinking water, mean serum Mg levels were increased and clinical incidence of hypomagnesemic tetany in beef breeding herds was decreased significantly. Similar results were observed by Rogers and Poole (1976). Reid et al. (1976) found that blood Mg concentrations varied markedly with the nature and Mg concentration of the basal hay diet when pregnant beef cows were supplemented with Mg as Epsom salts in the drinking water. Studies done by Reid et al. (1979) compared Mg supplementation through plant fertilization with supplementary Mg as Mg sulfate in drinking water. Both supplementation and fertilization resulted in significant increases in apparent absorption, Mg retention and serum Mg concentrations. The responses however were, markedly greater with Mg-sulfate added to water then with Mg fertilization. Similarly, Thompson and Reid (1981) noted that fertilization of orchard grass with kieseriete was effective

in increasing apparent absorption of Mg, Mg retention and serum Mg concentration in lactating beef cows and wether lambs. In a more recent experiment Reid et al. (1984) found that both kieseriete fertilization and addition of Epsom salts to drinking water of sheep resulted in increased serum Mg concentrations. Addition of Epsom salts to water increased intake of both fertilized and unfertilized hays but did not affect water intake.

While Mg-oxide is frequently used as a standard for comparison in bioavailability studies, it should be noted that different sources of the oxide may differ markedly in their dissolution and reaction rates in the gut. Bouwman (1978) compared the absorption and urinary excretions of several Mg-oxide sources and observed wide variations in availability among the Mg-oxide sources. Jensen et al. (1986) tested the reactivities of a number of commercial Mg-oxide products by pH-static titration and found the calcined magnesites to be significantly less reactive than Mg-oxide manufactured from dolomite. Particle size was an important factor although grinding of the coarser material did not always lead to the desired increase in reactivity because impurities produced silicic acids which inhibited dissolution. In vivo studies on the effect of Mg-oxide particle size on the solubility of Mg in the rumen have shown that the more finely ground Mg-oxides were more readily available for absorption. Jesse et al. (1981) studied availability of different sizes of Mg-oxide forms that were manufactured in the same type of calcining procedure.

Relative availability of Mg was measured by urinary excretion of Mg and milk fat production. Both criteria indicated that availability of Mg was higher for the smaller particles. However, at the smallest particle sizes (less then 57 um) the increase in urinary Mg excretion was transitory and rapidly returned to pre-treatment levels within 2 days. Wilson (1981) discussed problems with rapidly available Mg and stated that the transitory increase of urinary Mg excretions observed with finely ground Mg-oxide supplements was likely also to occur in serum Mg concentrations. This, however, may be a disadvantage since in ruminants diagnosed as hypomagnesemic it is desirable to maintain elevated serum Mg concentrations throughout the period of risk. An additional problem with rapidly available Mg is that high concentrations of Mg can increase ruminal pH.

CHAPTER III
COMPARISONS OF METHODS TO DETERMINE RELATIVE BIOAVAILABILITY OF Mg IN Mg OXIDES FOR SHEEP: EXPERIMENTS 1 AND 2

Introduction

Hypomanganesemic tetany is considered a serious nutritional problem resulting in considerable losses to livestock producers (Fontenot, 1980). Increasing intake of Mg by supplementing the diet with a readily available Mg source will usually prevent this disorder (Bell, 1983). Commercially available Mg sources can vary widely in biological availability depending on origin of the percursor, impurity concentration, degree of calcination (Wardle, 1983) and particle size (Jesse et al., 1981).

Several reviews are available concerning various methods and criteria which have been used to determine the biological availability of Mg sources (Thompson, 1965; Peeler, 1972; Reid, 1983) and which indicate their various advantages and disadvantages. Due to the expense and extremely short half-life (21 h) of the most commonly used radioisotope of Mg, determination of endogenous Mg excretion necessary to calculate true bioavailability is uncommon. Most feedstuffs contain more than adequate quantities of Mg to meet the requirements of growing ruminants, consequently semipurified diets which are expensive and often unpalatable must be used in experiments to determine Mg bioavailability at or below requirement levels.

A rapid, sensitive, inexpensive measure of bioavailability which does not necessitate the use of semipurified diets and radiotracers would be useful for the animal feed industry. Urinary Mg excretion immediately following high level supplementation may be a useful indicator. Urinary K concentration and daily urinary K excretion were suggested as measures of K bioavailability in young swine (Combs et al., 1985).

This study was undertaken to compare different methods of determining relative bioavailability (urinary Mg excretion vs Mg balance traits vs solubility) of several feed grade Mg oxides using reagent grade Mg sulfate as a standard reference source.

Materials and Methods

Two Mg balance trials were conducted with commercial Rambouillet crossbred wethers using a completely randomized design. Sheep were housed in individual metabolism cages designed for separate collection of urine and feces. In Experiment 1, a semi-purified, low Mg diet (200 mg/kg Mg (as-fed basis) by analysis; Table 1) was fed to 30 wethers averaging 46.7 kg initial weight. Following a 21-d depletion and adjustment period, wethers were assigned randomly to five experimental diets. Supplemental Mg was added to the basal diet at 600 mg/kg (as-fed basis) at the expense of cornstarch. Except for reagent grade $MgSO_4$ all other Mg supplements used in Experiment 1 were commercially available feed grade MgO sources (Table 2). For ease of identification, these sources will be referred to as oxides A, B, C and D. Feed intake was restricted to 800 g daily,

orts were measured daily and tap water (1.6 mg/kg Mg) was available ad libitum. Blood collections were made by jugular vein puncture at initiation and termination of the experimental period and serum frozen for Mg analysis.

During the 14-d experimental period, urine and feces were collected daily on the last 7-d. Urine was collected in open plastic buckets into which 100 ml of 3N HCl had been added to keep pH below 2. Urine volume was recorded daily and 10% aliquots were composited in plastic bottles and stored at 0 C until Mg analysis. Feces were collected in canvas collection bags, total fecal weights were recorded daily and 10% aliquots were composited and frozen in plastic bags for further analysis.

In Experiment 2, seven experimental diets were fed to 35 wethers averaging 44.3 kg initial weight. The basal diet was a conventional corn-soybean meal-cottonseed hulls diet (Table 3) which was formulated according to the recommendations of NRC (1985) and contained 1,387 mg/kg Mg (as-fed basis) by analysis. Dietary treatments were the basal diet supplemented with either 0, 700, 1,400 or 2,100 mg/kg Mg as reagent grade $MgSO_4$ or 1,400 mg/kg Mg from either oxide A, B or C. Feed intake was restricted to 1000 g daily. Sheep were fed the basal diet for a 10-d adjustment period then were switched to experimental diets for the 10-d trial. Urine was collected for 2 d at the end of the adjustment period and for 10 consecutive d during the experimental period. Fecal collections were made only during the last 4 d of the experimental period. Urine volume was recorded daily and 50 ml individual samples were saved in acid-washed

plastic bottles. Fecal collections were handled as described in Experiment 1. Blood was collected at the end of the adjustment period and d 10 of the experimental period and serum frozen.

Analyses of Mg in feed, urine, feces and serum was done by atomic absorption spectroscopy (Anonymous, 1982) on a Perkin-Elmer Model 5000 with AS-50 autosampler. Relative solubility of Mg sources in H_2O, .4% HCl, 2% citric acid and neutral ammonium citrate was determined after 1 h constant stirring on a stirring-hotplate at 37 C.

Data were analyzed using the general linear models procedure (GLM) of the SAS System (SAS, 1985). In Experiments 1 and 2, treatments were considered in a completely randomized design with animal the experimental unit. Treatment means were separated by Duncan's multiple range test. Regression analyses were performed by least squares using GLM for different traits to determine slope-ratios among different Mg sources. Daily urinary excretion time trends were analyzed using the repeated measures in time analysis of GLM with the Helmert option to determine when urinary Mg excretion for each treatment plateaued (SAS,1985). All possible subset regression using largest R-squared values was performed to determine relationships between Mg absorption or retention and daily urinary Mg excretion to select for the best combination of days during which to make urinary collections in future Mg bioavailability studies (SAS,1985). Data from one sheep fed the control, 700 mg/kg sulfate and oxide B diets were eliminated from calculation of Mg balance traits in Experiment 2 because of excessive (>50%) feed refusal on d 7 through d 10.

Results and Discussion

Physical and chemical characterization of the Mg sources used in Experiments 1 and 2 is found in Table 2. Origin of the reagent grade sulfate was unknown. Oxides A and D were derived from seawater and were similar products differing chiefly in particle size, with oxide A having smaller prills than oxide D. Oxide B was derived from brine and had a particle size distribution which was similar to that of oxide D. Oxide C was a calcined magnesite and was determined by x-ray diffraction analysis to have a greater degree of contaminants than other sources. Oxide C also had the smallest particle size distribution and was quite dusty. The large relative loss on ignition and lower MgO content for oxide C compared with other oxides appeared to be the result of considerable hydration. The chemical constituents of the oxides were determined by x-ray spectroscopy and supported results of x-ray diffraction, with oxide C having higher concentrations of CaO, SiO_2, Al_2O_3 and Fe_2O_3 than other sources.

The analyzed Mg concentration of $MgSO_4$ was 14.7%, and the oxides were 53.9, 54.4, 40.2 and 55.0% for A, B, C and D, respectively (Table 2). The lower Mg content for oxide C was consistent with increased hydration of the product during storage. Good separations were obtained for different Mg salts in 2% citric acid and neutral ammonium citrate. In neutral ammonium citrate, solubility for the oxides was lower compared to solubility in citric acid, whereas solubility for $MgSO_4$ remained the same. Solubility of the seawater product was greater for

smaller size particles (oxide A) than larger particles (oxide D). The larger size seawater product, however, was more soluble than the brine product of similar size. In .4% HCl, which is used by the feed industry as an indicator of availability, there was less separation among different oxides and solubility was highest. Solubility in HCl may tend to overrate the value of sources for ruminants.

Bouwman (1978) compared solubilities of Mg oxides with Mg absorption, and found greater correlation when HCl was the solvent. In the present experiments, solubility in HCl gave the same rank order of availability as other parameters, but the magnitude of differences was not expressed. Ammerman et al. (1972) reported differences in availability of reagent grade Mg sulfate compared with oxide or carbonate based on absorption or retention, but all sources were 97% or more soluble in HCl, citric acid or neutral ammonium sulfate but not water. Jesse et al. (1981) reported greater solubility in ruminal fluid of smaller MgO particles.

In experiment 1, Mg intake of sheep fed all sources was similar ($P > .10$; Table 4). Fecal Mg excretion of sheep fed oxides A, B and D was higher ($P < .01$) than that of sheep fed sulfate or oxide C which were similar. The amount of Mg absorbed from diets containing sulfate or oxide C was greater ($P < .01$) than that from oxide B or D, but oxide A was similar. Apparent absorption of Mg followed the same trend as total Mg absorption. Urinary Mg excretion was greatest for sheep fed oxides C and A and sulfate followed by oxides D and B ($P < .01$). Net retention

of Mg was not different among sulfate or oxide C but oxides A, B and D were lower ($P < .05$) compared to sulfate. Magnesium retention was negative for sheep fed oxides B and D, which had the largest size particles. When retention was expressed as a percentage of intake, the rank order was the same as that for net retention. When total Mg absorbed was used to express relative Mg bioavailability based on 100% for sulfate, values for oxides A, B, C and D were 58, 21, 89 and 33%, respectively. Based on values for absorption, the oxide of brine origin (B) was the least available of the sources tested. Although oxide C contained the largest amount of contaminating elements, which could be expected to possibly decrease availability, this oxide was the most available of the oxides. This MgO was of calcined magnesite (carbonate) origin and had the smallest particle size distribution of all sources. Although the seawater oxides (A and D) were essentially the same product, the larger particle distribution of oxide D reduced absorption and, consequently, availability of this product. The particle size of oxides B and D were similar as were their absorption and retention by sheep.

Calculations based on net retention were not done because of the negative values for oxides B and D. Bouwman (1978) also reported negative balance in sheep fed semipurified diets and supplemented with a similar amount of Mg from various sources. Chicco et al. (1972) reported positive balance in sheep when a semipurified diet containing a total of 250 to 700 mg of Mg was fed. Ammerman et al. (1972) reported negative Mg balance when 550 mg/kg Mg as raw magnesite was fed to sheep in a semipurified

diet, but reagent grade oxide, sulfate or carbonate maintained positive balances.

Final serum Mg concentration did not differ ($P > .10$) among treatments; however, sheep fed all Mg sources had greater ($P < .01$) final serum Mg concentrations compared with initial values (Table 4). The change in serum Mg tended to be greater for sheep fed oxide A than sheep fed other sources but differences were not significant. Serum Mg concentrations overrated the oxides compared with sulfate.

Jesse et al. (1981) was able to detect a difference in availability based on particle size of a single MgO source when serum Mg was used as a criterion. Chicco et al. (1972) reported a change in serum Mg concentration of approximately .10 mg/dl for each dietary addition of 150 mg of Mg as MgO after 7 d. Rahnema and Fontenot (1983) reported greater absorption and urinary excretion of Mg when MgO was fed to sheep compared with a similar amount of Mg from dolomitic limestone, but serum Mg concentrations were not different. Thomas et al. (1984) also reported differences in urinary Mg excretion but not serum Mg concentration when MgO of varying particle sizes was fed to dairy cows.

In experiment 2, supplementation of the basal diet with all Mg sources increased ($P < .01$) total Mg intake (Table 5). Magnesium intake increased from 1,387 to 3,227 mg/d as dietary supplementation increased from 0 to 2,100 mg/kg Mg. At a comparable level of supplementation (1,400 mg/kg), there was no difference ($P > .10$) in intake of Mg among the different

treatment groups. Supplementation of diets with graded levels of sulfate (700, 1,400 and 2,100 mg/kg) resulted in increased ($P < .01$) fecal Mg excretion. Feeding oxide B resulted in greater ($P < .05$) fecal Mg excretion compared with sulfate but oxide C and A were similar to sulfate. Absorption of Mg increased ($P < .01$) with increasing sulfate supplementation. Feeding 1,400 mg/kg Mg as oxide B resulted in Mg absorption which was not different from feeding control diets. Apparent absorption of Mg was lower ($P < .01$) when oxide B was fed compared with sulfate, but other oxides were similar. Urinary Mg excretion at D 7 to 10 of the experiment was similar for sheep fed 1,400 mg/kg Mg from all sources but increased ($P < .01$) as sulfate supplementation increased. Magnesium net retention was negative for sheep fed the control diet but this value was only lower ($P < .05$) than in sheep fed sulfate at 1,400 or 2,100 mg/kg. Retention expressed as a percentage of intake was relatively insensitive ($P > .10$) to dietary Mg supplementation.

Araujo (1976) reported negative retention for sheep fed approximately 4 to 5 g Mg daily as sulfate, hydroxide, carbonate or magnesite in a corn-soybean meal-cottonseed hulls diet. The negative balance values at elevated dietary concentrations as observed with sulfate in the present experiment, may be due to absorption velocity. Highly soluble Mg is absorbed from the rumen faster thereby causing a more rapid rise in plasma Mg concentration so that relatively more Mg will be excreted via kidney and net retention lower. Net retention is also calculated indirectly so that all errors involved with sampling

and analysis of diet, feces and urine are cumulative for this measure.

To determine the relative availability of the different sources used in Experiment 2, means and slope ratios were used. When means were compared to that for sulfate, which was set at 100%, relative values were 88, 64, and 88 for oxides A, B and C, respectively. Magnesium absorption (dependent variable) was regressed on Mg intake for sheep fed the control diet and 1,400 mg/kg of each source. The slopes derived for sulfate and oxides A, B and C were .404, .319, .149 and 319, respectively. Sulfate was equated to 100%, and resulting relative availability values were 79, 37 and 79% for respective sources. When net retention was used as the depentent variable, the same procedure resulted in relative availability values of 91, 29 and 83% of sulfate, respectively; while a comparison of means resulted in availability values of 89,16 and 79%, respectively. Although the magnitude varied from Experiment 1, the MgO of brine origin also had the lowest relative bioavailability in Experiment 2.

Final serum Mg concentration did not differ ($P > .10$) among treatments but final values were lower ($P < .05$) than initial values for sheep fed the control diet and greater ($P < .05$) for sheep fed oxide C (Table 5). The change in serum Mg concentration of sheep fed the control diet during the 10-d experimental period was similar to that of sheep fed oxide A or B but was lower ($P < .05$) than that of sheep fed other treatments. Differences among final serum Mg concentrations for sheep fed diets supplemented with Mg were not observed, indicating the lack

of sensitivity of this parameter when adequate dietary levels were fed. Regression analyses on data from sheep fed graded levels of sulfate were performed to compare relationships between different Mg balance traits (Table 6). The greatest R^2 values for regressions of Mg balance traits on total Mg intake were obtained with absorption and net retention (.89 and .86), respectively. All regressions were significant ($P < .001$). In the regression of different Mg balance traits on Mg absorption (Table 6), urinary excretion had the best fit to the linear model ($R^2 = .91$) followed by net retention ($R^2 = .82$). The high correlation ($r = .96$) between absorption and urinary excretion supports the suggestion that urinary excretion of Mg can be used to evaluate the availability of Mg in the diet. Similar findings were reported by Ammerman et al. (1972), Chicco et al. (1972) and Bouwman (1978) who found correlations of .94, .95 and .71, respectively between urinary Mg excretion and absorbed Mg in sheep.

Daily urinary Mg excretion during the 10-d experimental period is found in Table 7. Mauchly's test for sphericity was significant indicating the necessity of multivariate analysis of variance (SAS, 1985). There was an effect of time ($P < .01$) and a time X treatment interaction ($P < .05$) on daily urinary Mg excretion. Time had significant ($P < .001$) linear, quadratic and cubic order components. The d on which urinary Mg excretion reached a plateau was dependent on dietary treatment. In sheep fed the control diet or oxide B, there was no difference ($P > .10$) between urinary Mg excretion on D 1 and the mean of the

remaining days. Urinary Mg excretion was not different ($P > .10$) from the mean of remaining days on D 2 for sheep fed 700 or 1,400 mg/kg Mg from sulfate and D 7 for those fed 2,100 mg/kg. For sheep fed oxides A or C, excretion of urinary Mg was not different ($P > .10$) on D 3 compared with the mean of remaining days. With the exception of the greatest level of sulfate supplementation, urinary Mg excretion reached a plateau by D 4 after changing to experimental diets. For ease of calculation, Duncan's multiple range test was used to compare treatment means within individual days. There were no differences ($P > .10$) in urinary excretion of Mg prior to the change from the basal to experimental diets or on the first d following. On D 2, excretion of Mg was greater ($P < .05$) in sheep fed oxide C and sulfate at 1,400 or 2,100 mg/kg Mg compared with sheep fed the control diet. On D 3 urinary Mg excretion was also greater in sheep fed oxide C and sulfate than in sheep fed the control diet. On D 4 and 5, when urinary excretion had plateaued and stabilized, feeding oxide B resulted in excretion similar to the control diet. At the same level of dietary addition, Mg from sulfate and oxide A or C resulted in similar amounts of Mg in urine, but feeding oxide B resulted in lower ($P < .01$) urinary Mg excretion than sulfate or oxide C.

The means for urinary excretion on D 7 through 10 in Table 5 are not the same as those found in Table 7 as described in the experimental procedure. On D 7 to 10, sheep fed 2,100 mg/kg Mg excreted more ($P < .01$) Mg in urine than sheep fed other diets; however, there were no differences in urinary excretion of Mg

among sheep fed 1,400 mg/kg from any source. A better separation among sulfate levels was found for means in Table 7 than Table 5. The mean separation test of urinary Mg excretion on D 3, D 4 or D 5 was able to determine a difference between sheep fed sulfate and oxides fed at the same level but this difference was not detected for absorption and retention in the present experiment indicating that urinary Mg excretion shortly following high level Mg supplementation was more sensitive to dietary Mg than Mg balance traits presented in Table 5.

When sulfate was added to diets at graded levels, the regression of total daily urinary Mg excretion on added dietary Mg concentration indicated the best fit to a linear model occurred on D 4 followed by D 5 (R^2 = .04, .51, .54, .75 and .66 for D 1 through 5, respectively).

Regressions of daily urinary Mg excretion as the dependent variable against different Mg balance traits (Table 8) were used to determine the days on which urinary Mg excretion was most indicative of absorption and retention. All possible combinations of days from 1 to 5 were tested but only those combinations with consecutive days are presented in Table 8. Among the different parameters, the best R^2 values were obtained in the regression where absorption was used as the independent variable. When a single day's excretion was used in the model, D 4 had the greatest correlation between urinary excretion and all traits except apparent absorption. However, a minimum of two d should be used for bioavailability experiments in case a single day's collection is lost due to failure of some part of the

collection apparatus. When using two sequential days in the model, D 4,5 gave the greatest R^2 value for all traits except apparent absorption but the D 3,4 model was similar. No improvement was made when D 3 was added to the D 4,5 model, indicating that the extra d of collection would have little value. The slight improvement to the 2-d model by increasing to a 3,4 or 5-d model was probably the result of including the additional variables.

Based on the sensitivity of urinary Mg excretion to dietary Mg and good agreement between Mg absorption and urinary excretion, data suggested that urinary Mg excretion on D 4, 5 (data for each d is spearate, a mean of days is not used) following a change to diets containing 700, 1,400 and 2,100 mg/kg Mg could be used to predict the relative bioavailability of supplemental Mg sources. Shockey et al. (1984.) pursued the approach of using fecal Mg excretion to estimate Mg absorption. While the collection of uncontaminated fecal samples is easier than collection of urinary samples, there must be a 6 to 7 d time delay for fecal samples to reflect dietary intake before collections can begin, followed by a 5 to 7 d collection period. The urinary collection on D 4, 5 following feeding experimental diets is suggested for use in the present experiment. Therefore the experiment can be 6 to 9 d shorter by using urinary excretion to estimate absorption. Analysis of urinary Mg samples is also much more rapid than that of fecal samples, as they need only be diluted and concentration determined by atomic spectroscopy. Fecal samples must be ground, dried, ashed,

solubilized in acid and filtered prior to analysis, increasing laboratory time an additional 3 to 5 d.

The results indicated that the biological availability of feed grade Mg oxides varied considerably when compared on the basis of absorption and urinary excretion which is in agreement with results reported by other workers (Bouwman, 1978; Wilson, 1981; Jensen et al., 1986). An explanation for the observed differences in biological availability is the origin of the product and the method of fabrication used during production. Wilson (1981) demonstrated that dietary availability of calcined magnesite was correlated with the temperature used in the calcination process. Jensen et al. (1986) reported that calcination of Mg oxides, which might cause sintering of Mg crystals at high temperatures, resulted in decreased reactivity. Bouwman (1978) reported lower availability based on absorption, urinary excretion and retention of a "dead burned" Mg oxide from seawater compared with a "light burned" oxide.

There is also considerable evidence that the availability of Mg oxides is dependent upon particle size. Jesse et al. (1981) demonstrated that greater mesh size (smaller MgO particles) produced more rapid excretion of Mg in urine after oral administration of MgO. In another experiment, Jensen et al. (1986) found increased reactivity with decreasing particle size, demonstrating a negative relationship between Mg availability and larger particle size. Noller et al. (1986) demonstrated longer rumen retention time when coarse MgO was fed, compared with fine MgO. Magnesium from MgO with a smaller particle size was more

available to dairy cattle and promoted a greater milk fat percentage than a similar MgO with larger particles (Thomas et al., 1984). Wilson (1981) reported greater availability of smaller size particles of several Mg sources. Differences obtained in the overall availability in the present study were therefore, the mean effect of particle size, method of fabrication and origin. The Mg in larger particles of brine origin (oxide B) was less available for sheep than that of a similar or smaller size from seawater or calcined magnesite.

As indicated by Thompson (1965) in his review of techniques for measurement of mineral bioavailability, all methods have some disadvantages, and this is especially true for Mg. The short half-life and expense of the radiotracer Mg-28, needed to estimate endogenous losses to calculate true availability, limits its use by reseachers and the animal feed industry. Calculation of percentage apparent digestibility and net retention do not take endogenous losses into account, consequently, dietary Mg intakes below maintenance commonly result in negative values for these expressions (Thompson, 1965). As in the present studies, these values are unacceptable for bioavailability measures.

A comparison of possible methods determining relative bioavailability of the four oxides compared with sulfate is found in Table 9. Magnesium net retention in Experiment 1 was omitted from calculations because of negative values. The change in serum Mg concentration appears to overestimate the value of oxides in Experiment 1 and both over- and underestimate values in Experiment 2. In Experiment 1, urinary excretion overestimated

oxides A and C, while absorption underestimated oxides A, B and D. Values estimated by absorption in Experiment 1, when Mg concentrations near the requirement for sheep were fed, were similar to values based on solubility in neutral ammonium citrate.

In Experiment 2, estimates for oxide A based on various calculations with absorption, net retention and urinary excretion ranged from 60 to 91% of sulfate with an average of 79%. Oxide B ranged from 16 to 79% and averaged 45%. There was less variability with oxide C, which ranged from 74 to 98% and averaged 87%. These mean values are similar to that estimated from solubility in 2% citric acid. The power to detect true differences among sources by urinary excretion is lost as animals approach steady state conditions on D 7 to 10 compared with D 4,5. Based on solubility, oxide C would be expected to be more available than oxide A followed by oxide B. This difference was apparent from urinary excretion on D 4,5, but not mean absorption values, which failed to separate oxide A and C.

All of the approaches presented in Table 9 have been utilized in the literature and their use could be justified for various reasons. One method to calculate slope ratios would be to use all levels of sulfate; however, although absorption, retention and urinary excretion of Mg were linear with respect to dietary addition, all had higher order components when 2,100 mg/kg was included. Use of only the 0 and 1,400 mg/kg additions could thus be justified, which shifted the slope for sulfate and changed relative values from 1 to 7 percentage units for the oxides. For

absorption and urinary excretion, use of either slope ratio technique decreased the relative value of oxides compared to estimates based means, while those for net retention increased estimates for oxides. The idea that the pre-collection urinary excretion would be indicative of that which was being contributed by the basal diet and the difference at D 4,5 could be attributed to the source was tried. This calculation resulted in lower estimates for oxides A and B than the mean for D 4,5. As time increased from 1 to 10 d, urinary excretion of Mg in sheep fed the control diet also increased, indicating that steady state conditions had not been reached and body pools were still exchanging. The urinary excretion from sheep fed the control diet on D 4,5 was subtracted to account for that portion of the excretion from the basal diet. Relative values were again lower for oxides A and B, but results were generally similar to those calculated by absorption in Experiment 1.

The use of slope ratios to determine the relative availability of sources is only useful if the response of the dependent variable is highly linear within the interval at which the regression analyses are performed. Using slope ratio techniques to predict availability on data that are not linear or show limited relationships by regression analysis will be highly subjective to the chosen interval at which the slope was determined and therefore either overestimate or underestimate the true availability. This was observed when urinary excretion on D 1 or D 2 was used to calculate values for oxides B (4.9 and 39%) and C (33 and 57%) of sulfate, respectively.

When sources being compared with a highly linear response variable are similar in availability and thus have similar slopes, valid comparisons using means may be made at any level of dietary addition. However, if a highly available source such as sulfate is compared with a very unavailable source such as raw magnesite, slope ratios should be used for comparisons rather than mean values. The difference between mean values increases as dietary addition level increases. As an example, urinary excretion at D 4,5 varied 5 to 10 percentage units between the two methods of calculation for the more available oxides A and C, but varied 19 to 23 percentage units for less available oxide B.

Urinary Mg excretion at D 4 and 5 following supplementation of a natural diet with 1,400 mg/kg Mg was able to detect differences among Mg oxides of varying origin and particle size. Magnesium from MgO made from brine and having a larger particle distribution was less available than that from seawater or calcined magnesite with a smaller particle distribution.

Summary

Two Mg balance trials were conducted to compare relative bioavailability of Mg in several Mg oxides with that of reagent grade sulfate as determined by different methods. In Experiment 1, 600 mg/kg Mg (as-fed basis) as sulfate or four feed grade oxides varying in origin and particle size were added to a semipurified basal diet (200 mg/kg Mg, as-fed basis). Diets fed at 800 g/d to 30 crossbred wethers during the 14-d trial and fecal and urinary collections were made during the last 7 d. In Experiment 2, the basal corn-soybean meal-cottonseed hulls diet

(1,387 mg/kg Mg, as-fed basis) was supplemented with 0, 700, 1,400 or 2,100 mg/kg added Mg as reagent grade sulfate or 1,400 mg/kg Mg as three of the oxides from Experiment 1 and fed at 1,000 g/d to 35 wethers. Urine was collected daily for 10 d and feces were collected on D7 to 10. In Experiments 1 and 2, absorption of Mg from the oxide of brine origin and larger particle size distribution was lower ($P < .01$) than that from sulfate; but there was no difference ($P > .10$) in absorption in sheep fed oxides derived from seawater or calcined magnesite. In Experiment 2, urinary Mg excretion on D 4 and 5 of the collection was lower ($P < .01$) in sheep fed the brine oxide than those fed sulfate or MgO from calcined magnesite. Feeding the oxide from seawater resulted in urinary Mg excretion similar to that of sulfate. Data suggested that urinary Mg excretion on D 4 and 5 following addition of 1,400 mg/kg Mg to practical diets could be used to predict the bioavailability of Mg from Mg oxide sources.

TABLE 1. COMPOSITION OF THE SEMIPURIFIED DIET: EXPERIMENT 1

Ingredient	%[a]
Corn cobs, ground	35.0
Glucose monohydrate[b]	18.0
Cornstarch	29.8
Isolated soybean protein[c]	7.5
Urea	2.5
Corn oil[d]	3.0
Minerals[e]	3.2
Vitamins[f]	+
Cornstarch[g]	1.0

[a]As-fed basis. Contained 92.0% dry matter.

[b]Cerelose; Corn Products Co., Argo, IL.

[c]Supro 660; Purina Mills Inc., St. Louis, MO.

[d]Ethoxyquin was added to the diet at .0125% as an antioxidant; Monsanto Chemical Co., St. Louis, MO.

[e]The following reagent grade salts were added (% of diet): 1.13 $CaHPO_4$ $2H_2O$; 1.10 KCl; .929 Na_2SO_4; .063 Fe citrate; .0095 $ZnCO_3$; .0053 $MnCO_3$; .0020 $CuCl_2$; .00022 $CoCO_3$; .00018 KI.

[f]Vitamins added per kg of diet: 11 mg DL-alpha-tocopherol; 5,000 IU vitamin A palmitate; 1,000 IU vitamin D_3.

[g]Magnesium sources were added at the expense of cornstarch.

TABLE 2. PHYSICAL AND CHEMICAL CHARACTERIZATION OF MAGNESIUM SOURCES FED TO SHEEP: EXPERIMENTS 1 AND 2

Item	Sulfate (RG)	Oxide (FG) A	B	C	D
Origin	Unknown	Seawater	Brine	Calcined magnesite	Seawater
Compositionb	$MgSO_4$	MgO	MgO	Equal amounts of MgO & $Mg(OH)_2$, very minor $CaCO_3$ & traces of dolomite, quartz & olivine	MgO
Particle size, %					
+20	.1	.0	57.8	.9	52.3
+100	90.4	77.9	35.3	50.4	36.8
+200	9.2	9.2	.4	35.8	.2
-200	.3	12.9	6.5	12.9	10.7
Loss on ignition, %	NA[c]	1.21	.27	10.99	.42
Chemical constituents, %[d]					
MgO	NA	96.8	98.2	82.9	98.2
SiO_2	NA	.55	.41	1.18	.37
Fe_2O_3	NA	.25	.10	.70	.17
Al_2O_3	NA	.11	.14	.34	.06

TABLE 2. Continued

Item	Sulfate (RG)	Oxide (FG)			
		A	B	C	D
Cr_2O_3	NA	.02	.00	.00	.01
CaO	NA	.90	.86	3.74	.78
SO_3	NA	.19	.00	.18	.03
Cl	NA	.05	.00	.02	.00
Mg[e]	14.7	53.9	54.4	40.2	55.0
Relative solubility, %					
H_2O	99.6	.75	.09	1.16	.37
.4% HCl	100	88.9	81.8	89.4	79.0
2% Citric acid	100	76.6	40.5	86.7	51.1
Neutr. amm. citr.	100	45.5	20.1	85.8	38.1

[a]RG = reagent grade, FG = feed grade.

[b]By x-ray diffraction analysis (International Minerals and Chemical Corp., Mundelein, IL).

[c]NA = not analyzed.

[d]By x-ray spectroscopy (National Magnesia Chemicals, Moss Landing, CA).

[e]Analyzed percentage on the as-fed source following 4 h refluxing with 1:1 (v:v) $HCl-HNO_3$.

TABLE 3. COMPOSITION OF THE BASAL DIET: EXPERIMENT 2

Item	%[a]
Corn, ground	57.45
Cottonseed hulls	21.00
Soybean meal, dehulled	12.00
Alfalfa meal	3.00
Salt, trace-mineralized[b]	.50
Vitamins[c]	+
Ethyoxyquin	.0125
Limestone, ground	.55
Corn oil[d]	3.00
Cornstarch[e]	2.50

[a]As-fed basis. Contained 88.9% dry matter.

[b]Contained the following (%): 93 NaCl; .35 Zn; .28 Mn; .175 Fe; .035 Cu; .007 I; .007 Co.

[c]Vitamins added per kg of diet: 2,200 IU vitamin A palmitate; 440 IU vitamin D_3.

[d]Ethoxyquin was added to the diet at .0125% as an antioxidant (Monsanto Chemical Co., St. Louis, MO).

[e]Magnesium sources were added at the expense of cornstarch.

TABLE 4. EFFECT OF MAGNESIUM SOURCES ON MAGNESIUM BALANCE AND SERUM MAGNESIUM CONCENTRATION IN SHEEP: EXPERIMENT 1

Magnesium	Sulfate	Oxide A	Oxide B	Oxide C	Oxide D	SEM
Intake, mg/d	636	636	605	636	635	5.51
Fecal excretion, mg/d	398^{d}	498^{bc}	556^{b}	426^{cd}	557^{b}	14.6
Absorption, mg/d	238^{b}	137^{bc}	49^{c}	211^{b}	78^{c}	13.3
Apparent absorption, %	37.5^{b}	21.6^{bc}	8.1^{c}	33.2^{b}	12.3^{c}	2.14
Urinary excretion, mg/d	110^{bc}	128^{b}	57^{d}	154^{b}	79^{cd}	4.86
Net retention, mg/d	128^{e}	9.1^{f}	-7.7^{f}	57.3^{ef}	-1.3^{f}	12.9
Retention, % intake	20.19^{e}	1.47^{ef}	-1.04^{f}	9.05^{ef}	$-.16^{f}$	2.06
Final serum, mg/dl	2.12	2.25	2.03	2.22	2.14	.073
Change serum, mg/dl	.55	.91	.64	.62	.61	.048

[a]Each value represents the mean of six sheep. Magnesium sources added at 600 mg/kg to a basal diet that contained 200 mg/kg Mg (as-fed basis).

[b,c,d]Means in the same row without a common superscript differ ($P < .01$).

[e,f]Means in the same row without a common superscript differ ($P < .05$).

TABLE 5. EFFECT OF MAGNESIUM SOURCE ON MAGNESIUM BALANCE AND SERUM MAGNESIUM CONCENTRATION IN SHEEP: EXPERIMENT 2[a]

	Supplemental Mg, mg/kg							
	Control	Sulfate			Oxide A	Oxide B	Oxide C	
Magnesium	0 (4)[b]	700 (4)	1,400 (5)	2,100 (5)	1,400 (5)	1,400 (4)	1,400 (5)	SEM
Intake, mg/d	1,387[c]	2,087[d]	2,787[ef]	3,227[f]	2,787[ef]	2,787[ef]	2,708[ef]	41.0
Fecal excretion, mg/d	957[c]	1,364[d]	1,791[e]	1,843[ef]	1,910[ef]	2,147[f]	1,831[ef]	29.6
Absorption, mg/d	430[c]	723[cd]	996[de]	1,385[e]	877[d]	639[cd]	878[d]	36.2
Apparent absorption, %	31.0[cd]	34.6[cd]	35.7[c]	42.3[e]	31.4[cd]	22.9[d]	32.1[cd]	1.0
Urinary excretion, mg/d	474[c]	598[c]	761[cd]	1,016[d]	666[c]	601[c]	691[c]	24.4
Net retention, mg/d	-43.5[g]	124[gh]	235[hj]	369[j]	210[ghj]	38[gh]	186[ghj]	29.5
Retention, % intake	-3.14	5.95	8.44	10.77	7.54	1.36	6.29	1.1
Final serum, mg/dl	2.02	2.20	2.40	2.57	2.11	2.25	2.44	.10
Change serum, mg/dl	-.17[g]	.19[h]	.26[h]	.36[h]	.048[h]	.118[h]	.32[h]	.07

[a]Based on fecal and urinary collections from D 7 through 10.

[b]Control diet contained 1,387 mg/kg Mg (as-fed basis). Number in parentheses represents the number of sheep/treatment.

[c,d,e,f]Means in the same row without a common superscript differ ($P < .01$).

[g,h,j]Means in the same row without a common superscript differ ($P < .05$).

TABLE 6. LINEAR REGRESSION OF VARIOUS MAGNESIUM BALANCE TRAITS ON MAGNESIUM INTAKE AND MAGNESIUM ABSORPTION IN SHEEP SUPPLEMENTED WITH MAGNESIUM SULFATE: EXPERIMENT 2

Dependent variable	Equation	R^{2a}	SD[b]	PR>F
Absorption, mg/d	$Y = -333 + .511x$[c]	.89	144	.0001
Apparent absorption, %	$Y = 22.4 + .00567x$[c]	.45	4.9	.0023
Urinary excretion (D 7-10), mg/d	$Y = 38.8 + .284x$[c]	.71	143	.0001
Net retention, mg/d	$Y = -369 + .227x$[c]	.86	73	.0001
Retention, % intake	$Y = -11.7 + .00724x$[c]	.71	3.7	.0001
Urinary excretion (D 7-10), mg/d	$Y = 190 + .590x$[d]	.91	81	.0001
Net retention, mg/d	$Y = -190 + .410x$[d]	.82	82	.0001
Retention, % intake	$Y = -5.80 + .0128x$[d]	.65	3.9	.0001

[a]Coefficient of determination.

[b] Residual MS.

[c]Where Y equals the dependent variable and x equals Mg intake from sulfate, mg/d.

[d]Where Y equals the dependent variable and x equals Mg absorbed from sulfate, mg/d

TABLE 7. EFFECT OF MAGNESIUM SOURCE ON DAILY URINARY MAGNESIUM EXCRETION BY SHEEP: EXPERIMENT 2

	Supplemental Mg, mg/kg						
	Control	Sulfate			Oxide A	Oxide B	Oxide C
Time,[b] d	0[c]	700	1,400	2,100	1,400	1,400	1,400
Pre	310 ± 63	282 ± 49	242 ± 33	235 ± 18	302 ± 56	253 ± 51	236 ± 37
1	356 ± 39[x]	340 ± 61	517 ± 88	431 ± 66	470 ± 37	424 ± 78[x]	410 ± 41
2	353 ± 69[d]	472 ± 80[dex]	733 ± 25[fx]	767 ± 92[f]	553 ± 60[def]	467 ± 79[de]	597 ± 55
3	339 ± 65[d]	507 ± 84[ghj]	737 ± 43[kl]	855 ± 100[l]	625 ± 57[hjkx]	471 ± 73[gh]	676 ± 30
4	347 ± 61[d]	504 ± 41[hj]	747 ± 45[jl]	855 ± 75[l]	604 ± 42[hj]	493 ± 75[gh]	699 ± 22
5	358 ± 76[d]	572 ± 80[gh]	700 ± 45[jl]	884 ± 93[l]	625 ± 38[hj]	476 ± 78[gh]	706 ± 28
6	345 ± 66	540 ± 122	765 ± 48	914 ± 41	679 ± 41	525 ± 53	690 ± 42
7	456 ± 88	491 ± 113	769 ± 54	999 ± 95[x]	681 ± 56	594 ± 70	656 ± 74
8	504 ± 61	499 ± 80	745 ± 29	1,083 ± 167	638 ± 60	534 ± 48	708 ± 73
9	404 ± 66	558 ± 103	824 ± 68	1,014 ± 151	666 ± 45	565 ± 73	722 ± 94
10	409 ± 65	531 ± 84	800 ± 76	989 ± 101	678 ± 30	502 ± 46	672 ± 71
Mean D 7-10	405[d]	520[gh]	785[hj]	1,021[j]	666[gh]	549[gh]	690[gh]

[a]Each value represents the mean ± standard error of five sheep.

[b]Time after feeding experimental diets. Pre represents the mean from the 2 d prior to feeding experimental diets.

[c]Control diet contained 1,387 mg/kg Mg (as-fed basis).

[d,e,f]Means within rows without a common superscript differ (P < .05).

[g,h,j,k,l]Means within rows without a common superscript differ (P < .01).

[x]Within a dietary treatment, no difference (P > .10) between this day and the mean of the remaining days.

TABLE 8. R-SQUARE VALUES FOR REGRESSION MODELS OF URINARY EXCRETION BY DAY AND MAGNESIUM BALANCE TRAITS: EXPERIMENT 2[a]

	R-square			
Variables in model	Absorption, mg/d	Apparent absorption, %	Retention, mg/d	Retention, % intake
Day 1	.047	.073	.003	.002
Day 2	.701	.597	.409	.328
Day 3	.815	.665	.477	.397
Day 4	.863	.611	.518	.430
Day 5	.699	.564	.394	.350
Day 1,2	.748	.611	.493	.398
Day 2,3	.825	.680	.483	.399
Day 3,4	.864	.665	.517	.430
Day 4,5	.871	.615	.533	.433
Day 1,2,3	.882	.699	.578	.480
Day 2,3,4	.866	.682	.518	.430
Day 3,4,5	.876	.666	.535	.434
Day 1,2,3,4	.910	.704	.597	.497
Day 2,3,4,5	.876	.683	.538	.435

[a]Absorption and retention calculated from samples collected on D 7 through 10. Represents data from 32 sheep (Table 5).

TABLE 9. RELATIVE BIOAVAILABILITY OF MAGNESIUM SOURCES CALCULATED BY DIFFERENT METHODS: EXPERIMENTS 1 AND 2

Method of calculation	Sulfate	Oxide A	Oxide B	Oxide C	Oxide D
			Exp. 1		
Absorption mean[a]	100	58	21	89	33
Urinary excretion mean[a]	100	116	52	140	72
Serum change mean[a]	100	165	116	113	109
			Exp. 2		
Absorption mean[b]	100	88	64	88	---
Absorption (slope ratio)[c]	100	79	37	79	---
Absorption (slope ratio)[d]	100	74	36	74	---
Net retention mean[b]	100	89	16	79	---
Net retention slope ratio[c]	100	91	29	83	---
Net retention slope ratio[d]	100	90	26	81	---
Urinary excretion mean D 7-10 (Table 5)[b]	100	88	79	91	---
Urinary excretion mean D 7-10 (Table 7)[b]	100	85	70	88	---
Urinary excretion mean D 4,5[b]	100	80	63	96	---
Urinary excretion slope ratio D 4,5[c]	100	70	40	91	---
Urinary excretion slope ratio D 4,5[d]	100	75	44	98	---

TABLE 9. Continued

Method of calculation	Sulfate	Oxide A	Oxide B	Oxide C	Oxide D
Urinary excretion (mean - pre) D 4,5[b]	100	60	44	95	---
Urinary excretion (mean - control) Day 4,5[b]	100	64	32	92	---
Serum change mean (mg/dl)[b]	100	15	42	123	---
Relative solubility, %					
H^2O	100	<1	<1	1	<1
.4% HCl	100	89	82	89	79
2% Citric acid	100	77	41	87	51
Neutral ammonium citrate	100	45	20	85	38

[a]Based on means of groups fed 600 mg/kg added Mg from all sources. Basal semipurified diet contained 200 mg/kg Mg (as-fed basis).

[b]Based on means of groups fed 1,400 mg/kg added Mg from from all sources. Basal practical-type diet contained 1,387 mg/kg Mg (as-fed basis).

[c]Based on slope ratio calculated from 0 and 1,400 mg/kg added Mg from all sources.

[d]Based on slope ratio calculated from 0, 700, 1,400 and 2,100 mg/kg added Mg from sulfate and 0 and 1,400 mg/kg from oxides.

CHAPTER IV
MEASUREMENTS OF Mg AVAILABILITY IN RAW MAGNESITE AND Mg-SULFATE: EXERIMENT 3

Introduction

Hypomanganesemic tetany is considered a serious nutritional problem resulting in considerable losses to livestock producers (Fontenot, 1980). Even though the etiology of grass tetany is not fully understood, there are a number of measures that can be taken to reduce losses. Increasing intake of Mg by supplementing the diet with a readily available Mg sources will usually prevent this disorder (Bell, 1983).

More than 60 Mg-containing compounds are known and can be classified into three groups: 1) carbonates (magnesite and dolomite) 2) salts and double salts found in oceanic deposits (carnallite, keiserite, langbeinite and epsomite) and 3) silicates (olivine, serpentine, talc) (Wardle, 1983). Commercial recovery of MgO from the silicates is not underway in the U.S. at the present time. Commercially available Mg sources can vary widely in biological availability depending on origin of the percursor, impurity concentration, degree of calcination (Wardle, 1983) and particle size (Jesse et al., 1981).

The amount of a mineral element in a particular compound has little meaning unless it is qualified with a correction factor indicating its degree of biological availability for a specie of animal. The term "biological availability" generally indicates

the degree to which an element is absorbed and available for use in physiological processes (Peeler, 1972). Several reviews are available concerning various methods and criteria which have been used to determine the biological availability of Mg sources (Thompson, 1965; Peeler, 1972; Reid, 1983) and which indicate their various advantages and disadvantages. Due to the expense and extremely short half-life (21 h) of the most commonly used radioisotope of Mg, estimation of endogenous Mg excretion which is necessary to calculate "true bioavailability" is rare. Because most feedstuffs contain more than adequate quantities of Mg to meet the requirements of growing ruminants, semipurified diets which are expensive and often unpalatable have been used in experiments to determine bioavailability at or below requirements.

A rapid, sensitive, inexpensive measure of bioavailability would be useful for the animal feed industry, and urinary excretion immediately following high level supplementation may be a useful indicator. This study was undertaken to compare different methods of determining relative bioavailability of raw magnesite by using reagent grade Mg sulfate as a standard reference source.

Materials and Methods

Thirty-six crossbred wethers averaging 41 kg initially were housed in individual metabolism crates designed for separate collections of urine and feces. For the duration of the experiment, sheep were fed a restricted diet of 1000 g daily. Tap water was available ad libitum. During a 10-d adjustment

period, all sheep were fed the basal corn-soybean meal-cottonseed hulls diet (Table 11) containing 1275 mg/kg Mg (dry basis) by analysis and formulated to meet the nutrient requirements of sheep (NRC, 1985). At the end of the adjustment period, blood samples were obtained by jugular vein puncture from all animals.

At initiation of the experimental period the sheep were assigned randomly to dietary treatments which included the basal diet supplemented with 0, 1000, 2000 and 3000 mg/kg Mg as sulfate or 1000 and 3000 mg/kg as raw magnesite. Final dietary levels were confirmed by chemical analysis. At the end of the 10 day experimental period, blood samples were collected as described previously.

Fecal and urinary collections were made daily throughout the 10-d experimental period. Collection procedures were identical to the procedures used in Experiments 1 and 2 which were described previously. Fecal, feed and urinary Mg concentrations were determined by flame atomic absorption spectrophotometry on a Pelkin-Elmer model 5000. Standards were matched for macroelement and acid concentrations and standard reference material from the National Bureau of Standards was included with the samples. Data were analyzed by using the general linear models procedure (Proc GLM) of the Statistical Analysis System (SAS, 1985). Treatments were separated by Duncan's Multiple Range Test. Regression analysis was performed for different parameters to determine slope-ratios among different Mg-sources. Daily urinary excretions were analyzed by using the Helmert option of the GLM procedure to determine at which day the urinary Mg concentrations

for each treatment plateaus. Proc R-square analysis was performed to determine correlations between Mg-absorption and daily urinary Mg excretions in order to select for the best combination of days to make urinary collections which are highly indicative of Mg availability.

Results

The magnesite source in this experiment, which was analysed by x-ray diffraction analysis, had equal amounts of MgO and $Mg(OH)_2$ with traces of calcium carbonate, dolomite, quartz and olivine (Table 10). The Mg concentrations, analyzed by atomic absorption were 14.7 percent and 40.2 percent for the sulfate and magnesite sources, respectively. Chemical constituents, CaO, Al_2O_3 and Fe_2O_3, as determined by x-ray spectroscopy, were relatively high magnesite. Particle size was homogenous for 99.9% of particles. Relative solubilities for magnesite did not vary greatly among the different solvents (Table 10) and were highest in .4% hydrochloric acid (89.4%) followed by the solubilities in 2% citric acid (86.7%) and ammonium citrate (85.8%). The final serum Mg concentration (Table 12) was higher ($P < .05$) for sheep fed Mg-sulfate at 3000 mg/kg when compared to those for other treatments. All treatments showed increased ($P < .05$) final serum for Mg concentrations when compared to initial values. Increases in serum Mg were greatest ($P < .05$) for sheep fed the Mg-sulfate at the 2000 and 3000 mg/kg intake level.

Increasing dietary supplementation from 0 to 3000 mg/kg, increased the total daily Mg intake per sheep from 1275 mg to

4307 mg (Table 12). Fecal Mg excretions increased linearly (R^2 = .91) with increasing dietary supplementation (Table 13). At the 3000mg/kg level of supplementation fecal Mg excretions for the sheep fed magnesite were higher ($P < .05$) than those for sulfate, but when compared at the 1000 mg/kg supplementary level, no differences ($P < .05$) were observed (Table 12). Absorption of Mg (Table 13) increased linearly with dietary supplementation (R^2 = .81) and followed the same trend as fecal Mg excretions. At comparable levels of supplementation, no differences in Mg absorption were observed at 1000 mg/kg level, while at the 3000 mg/kg supplementary level, Mg as sulfate was better absorbed ($P < .05$) than that from magnesite. When absorption was expressed as a percent of total Mg intake (apparent absorption) Mg absorption was higher ($P < .05$) for sulfate at the 3000 mg/kg level than the 1000 mg/kg level. When comparisons were made between sources that for apparent absorption from magnesite was lower ($P < .05$) than sulfate at the 3000 mg/kg level, but did not differ at the lower level of supplementation (1000 mg/kg). Urinary Mg excretion (Table 13) increased linearly with dietary Mg supplementation (R^2 = .81). When urinary Mg excretions were compared between sources, excretions for Mg or sulfate were higher at both the 1000 and 3000 mg/kg supplementary level. Comparison of urinary Mg within each source showed that Mg excretion was not different for magnesite at different supplementary levels while urinary Mg excretion were higher for sheep receiving 3000 mg/kg as sulfate when compared to Mg excretion of sheep receiving 1000 mg/kg Mg as sulfate.

Retention of Mg (Table 13) showed no linear trent (R^2 = .39) with increases in dietary supplementation. At the 3000 mg/kg level, Mg retention was not different ($P < .05$) between the sulfate and magnesite but was higher ($P < .05$) for magnesite when comparisons were made at the 1000 mg/kg supplementary level (Table 12). The Mg retention value for sulfate at 1000 mg/kg was negative. When retention was expressed as a percent of intake (Ret. % I), the same trend was observed. Regression analysis (Table 13) performed on data from sheep supplemented with reagent grade sulfate showed that absorption, urinary excretion and fecal excretions increased linearly with dietary intake. The best fit to the linear model was observed for fecal Mg excretion (R^2 = .91). Response of apparent Mg absorption, Mg retention and retention % I was not linear as indicated by the low R-square values (Table 13) when regressed against Mg intake. When regressions of Mg absorption and different Mg balance parameters were used (Table 13), excretion and retention showed good correlations (R^2 = .63 and .58, respectively) while the best correlation was found for Mg absorption and urinary Mg excretion (R^2 = .79).

The slope ratio technique was used to compare relative availability of the magnesite source. Slopes from the regressions of Mg intake and Mg absorption were used for this purpose and were .459 and .232 for sulfate and magnesite, respectively. Equating the sulfate to 100% the relative availability of magnesite was : 50.5 percent.

To determine which day or which consecutive days of urinary Mg excretions were most indicative for bioavailability measurements, regression models of daily urinary excretions and balance parameters were used. The R-square values were used as criteria to select the best days. With single day's excretion in the model the highest R-square value was obtained with day 5 when Mg absorption was used as the independent variable. When, retention, retention % I and apparent absorption were used as independent variables, the highest R-square values were obtained with day 4. However, regressions between absorption and urinary Mg excretion resulted in R-square values that were superior over all other parameters used. Using 2 consecutive days as a variable in the model, the best combination was obtained with day 4, 5 for all different parameters used as dependent variables. When 3 days were used in the model the highest R-square value was obtained with day 4, 5 and 6. However, little improvement was obtained with using 3 consecutive days as a variable in the model. Daily urinary Mg excretions during the 10-d experimental period are found in Table 16. To determine the number of days after which urinary Mg excretions plateaued for each treatment the Helmert option of the GLM procedure was used. Results from this test, indicated that sheep, receiving diets supplemented with raw magnesite, had no significant increases in urinary Mg concentration throughout the 10-d collection period. This was also true for sheep receiving the control diet. Using a P-value of .05 as the significance level to determine at which day the urinary Mg excretions plateaued, results showed that urinary Mg

excretions of sheep receiving the 2000 mg/kg sulfate diets plateaued at day 2, while for sheep receiving the 3000 mg/kg Mg-sulfate supplemented diets, urinary Mg excretions plateaued at day 3. Table 15 shows the results of the relative availabilites of Mg using different methods of calculation. Using the mean urinary excretion of day 4, 5 to calculate the Mg availability, the following results were obtained: at the 1000 mg/kg supplementary level, relative availabilities were 100 vs. 51 percent for sulfate and raw magnesite, respectively. When calculations were based on urinary Mg excretions of day 4, 5 at the 3000 mg/kg supplementary level the availability of raw magnesite was lower (31%). Using the relative increase in urinary Mg concentrations over the control treatment the relative Mg availabilities for magnesite at both the 1000 and 3000 mg/kg supplementary level were negative indicating a very poor utilization of Mg in raw magnesite.

Discussion

The dietary levels chosen in this experiment ranged from 0 to 3000 mg/kg Mg. When using the technique where dietary Mg levels are fed in excess of the requirement, it is important that animal performance is not affected by high levels of supplementation. In the present experiment, cases of diahrrea were observed in sheep receiving the 3000 mg/kg Mg-sulfate diets indicating that the maximum tolerable level was exceeded. It is therefore recommended that lower Mg concentration, especially with highly available Mg-salts, should be used in future Mg bioassays.

When Mg absorption was used as criteria for bioavailability, the calculated availability for raw mangnesite (50.5 percent) was low when compared to results obtained with the solubility tests. The solubility of Mg from raw magnesite ranged from 85.8 percent to 89.4 percent when tested in different solvents. The solubility tests therefore tended to over estimate the biological availaibility of Mg in raw magnesite. This is not in agreement with the results obtained in earlier experiments and the results reported by Bouwman (1978) where good correlations were obtained between solubilities and relative availabilities for different Mg oxides. In these experiments, however, most of the Mg oxides tested were quite available (availability was greater than 70%) whereas the Mg in raw magnesite was utilized very poorly.

Increases in the total Mg absorbed due to increased dietary concentrations were also observed in the earlier experiments and clearly indicate that no homeostatic mechanism was in control to restrict absorption at the higher Mg intakes. This is an important consideration in studies where the relative availability of a particular source is based upon absorption of a element that is fed in excess of the requirement. Homeostatic control will affect absorption and will therefore underestimate the relative availabilities of highly available Mg sources.

Fecal mg excretions were highly correlated (R^2 = .91) with Mg intakes. Since fecal Mg consists of mostly unabsorbed Mg from the diet, the high correlation between fecal Mg excretion and Mg intake, indicates that this parameter can also be used as an indicator of unavailable Mg. Shokey et al., (1984) observed

similar results and used fecal Mg excretions as a predictor of Mg bioavailability. As already observed in earlier experiments, retention was insensitive to dietary Mg supplementation and was therefore not suitable for use as an indicator of bioavailability. Similar results were also reported by Bouwman (1978) and Araujo (1976).

The good correlation between urinary Mg excretion and absorption again indicates the usefulness of urinary Mg excretions as an indicator of Mg bioavailability. Good correlations of urinary Mg excretion and Mg absorption were also obtained in earlier experiments and agrees with findings of Ammerman et al., (1972), Chicco et al., (1972) and Bouwman (1978). The poor response in urinary Mg concentrations due to dietary supplementation with raw magnesite indicates the low Mg availability in this product.

The use of slope ratio techniques to determine the relative availability of a particular source is only usefull if the response of the dependent variable is highly linear within the interval at which the regression analysis is performed. Using slope ratio techniques on data that are not linear or show low correlations in regression analysis to predict availability will be highly subjective to the chosen interval at which the slope was determined and therefore either underestimate or overestimate true availability. In contradiction to the regressions of Mg absorption and intake, the observed increases in urinary Mg excretions from day 0 to day 3 were not linear and are therefore unsuitable for the use of slope ratio technique. More valid

comparisons can be made after the Mg concentrations in the urine reach optimum levels (plateaus) and remain stable for some period. In the present experiment, all urinary Mg excretions plateaued not later then day 3 and therefore comparisons between sources are more valid when made after day 3. When measuring increases of urinary Mg output over the control treatment for day 5, the relative availabilities for Mg as magnesite were negative, again indicating a very low availability for this form. As was indicated by the Helmert procedure, urinary Mg excretion of sheep fed both 1000 and 3000 mg/kg as raw magnesite did not increase over time and did not differ from the control group. This indicates that despite the dietary supplementation up to levels of 3000 mg /kg urinary Mg excretion was not affected. Based upon this observation it seems that the best indication of relative availability was obtained when increases of urinary Mg excretion over excretion for the control group were used in the calculations, i.e., 0 percent availability.

Summary

Thirty-six crossbred wethers were used to measure the relative Mg availabilities of reagent grade sulfate and raw magnesite. During the 10-day adjustment phase all sheep were fed the basal diet (corn-soybean meal-cottonseed hulls) and placed in individual raised slotted pens designed for separate collections of urine and feces. Dietary treatments included the basal diet supplemented with either 0, 1000, 2000 or 3000 mg/kg Mg as reagent grade sulfate or 1000 or 3000 mg/kg Mg as raw magnesite. Feed intake was restricted to 1000 grams daily and tap water was

available ad libitum. At initiation and termination of the experimental period blood samples were obtained by jugular vein puncture. Data indicated that Mg absorption increased linearly for sheep fed Mg as sulfate. Magnesium absorption did not increase ($P < .05$) for sheep fed 1000 mg/kg Mg as raw magnesite. Using dietary supplementations of 3000 mg/kg Mg as raw magnesite only a slight increase in absorption was observed. The same trend was observed with urinary Mg excretion; increases were linear for sheep fed Mg as reagent grade sulfate and no change in urinary Mg excretion was observed for sheep fed either 1000 or 3000 mg/kg Mg as raw magnesite. Calculations on the percent availability varied with the method of calculation used; with the slope ratio technique, the Mg availability for sulfate and raw magnesite were 100 and 50.5 percent, respectively. Using mean urinary Mg excretions of day 4,5 at the 1000 mg/kg supplementary level the relative availability for raw magnesite was 51 percent. At the 3000 mg/kg supplementary level the relative availability for magnesite was 31 percent. Increases in urinary Mg excretion over the control treatment resulted in negative availabilities for magnesite indicating a poor utilization of Mg in raw magnesite.

TABLE 10. PHYSICAL AND CHEMICAL CHARACTERIZATION OF MAGNESIUM SOURCES FED TO SHEEP: EXPERIMENT 3

Item	Sulfate	Magnesite
Origin	Unknown	Raw Magnesite
Composition	$MgSO_4$	MgO; Equal amounts of MgO and $Mg(OH)_2$, traces of $CaCO_3$, dolomite quartz and olivine
Particle size, %		
+20	.1	--
+100	90.4	--
+200	9.2	--
-200	.3	--
Loss ignition, %	NA	--
Chemical constituents, %		
MgO	NA	--
SiO_2	NA	--
Fe_2O_3	NA	--
Al_2O_3	NA	--
Cr_2O_3	NA	--
CaO	NA	--
SO_3	NA	--
Cl	NA	--
Mg	NA	--
Relative solubility, %		
H_2O	99.6	<1
.4% HCl	100	89.4
2% citric acid	100	86.7
Neutral ammonium citrate	100	85.8

TABLE 11. COMPOSITION OF THE BASAL DIET: EXPERIMENT 3

Item	%[a]
Corn, ground	57.45
Cottonseed hulls	21.00
Soybean meal, dehulled	12.00
Alfalfa meal	3.00
Salt, trace-mineralized[b]	.50
Vitamins[c]	+
Ethyoxyquin	.0125
Limestone, ground	.55
Corn oil[d]	3.00
Cornstarch[e]	2.50

[a]As-fed basis. Contained 88.9% dry matter.

[b]Contained the following (%): 93 NaCl; .35 Zn; .28 Mn; .175 Fe; .035 Cu; .007 I; .007 Co.

[c]Vitamins added per kg of diet: 2,200 IU vitamin A palmitate; 440 IU vitamin D_3.

[d]Ethoxyquin was added to the diet at .0125% as an antioxidant (Monsanto Chemical Co., St. Louis, MO).

[e]Magnesium sources were added at the expense of cornstarch.

TABLE 12. EFFECT OF MAGNESIUM SOURCE ON MAGNESIUM BALANCE AND SERUM MAGNESIUM CONCENTRATION IN SHEEP: EXPERIMENT 3[a]

	Control	Sulfate			Magnesite	
Magnesium	0 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg	1000 mg/kg	3000 mg/kg
Intake, mg	1275.0 ±31.6^{d}	2307.0 ± 0.0^{c}	3307.0 ± 0.0^{b}	4307.0 ± 0.0^{a}	2307.0 ± 0.0^{c}	4307.0 ± 0.0^{a}
Feces, mg	759.0 ±54.1^{e}	1517.0 ±93.1^{d}	1899.0 ±42.1^{c}	2438.0 ±128.1^{b}	1663.0 ±42.3^{d}	3199.0 ±63.4^{a}
Absorbed, mg	515.0 ±36.8^{e}	790.0 ±93.1^{d}	1408.0 ±42.1^{b}	1868.0 ±128.1^{a}	644.0 ±42.3^{de}	1107.0 ±63.4^{c}
Apparent absorption, %	40.6 ± 3.2^{ab}	34.2 ± 4.0^{bc}	42.6 ± 1.3^{a}	43.4 ± 2.9^{a}	27.9 ± 1.8^{cd}	25.7 ± 1.5^{d}
Urinary, mg	427.0 ±54.1^{c}	826.0 ±84.4^{b}	1356.0 ±46.5^{a}	1307.0 ± 54.7^{a}	448.0 ±48.7^{c}	431.0 ±41.4^{c}
Retained, mg	88.7 ±42.7^{bc}	-37.1 ±50.5^{c}	52.0 ±28.4^{bc}	560.8 ±120.8^{a}	195.4 ±29.0^{b}	676.0 ±28.6^{a}
Retained, % intake	7.4 ± 3.8^{bc}	-1.6 ± 2.2^{d}	1.6 ± 0.9^{cd}	13.0 ± 2.8^{ab}	8.5 ± 1.3^{abc}	15.7 ± 0.66^{a}
Final serum Mg, mg/dl	2.35 ± 0.09^{b}	2.56 ± 0.13^{b}	2.64± 0.16^{b}	3.0 ± 0.15^{a}	2.3 ± 0.04^{b}	2.28 ± 0.09^{b}
Change serum Mg, mg/dl	0.232± 0.07^{bcd}	0.404± 0.09^{bc}	0.56± 0.13^{ab}	0.87± 0.16^{a}	0.02± 0.07^{d}	0.086± 0.12^{cd}

[a]Control diet contained 1275 mg/kg Mg (as-fed basis). Based on fecal and urinary collections from 7-10d.

^{b,c,d,e,f}Means in rows with different superscripts differ (P<.05).

TABLE 13. LINEAR REGRESSION OF VARIOUS MAGNESIUM BALANCE PARAMETERS ON MAGNESIUM INTAKE AND MAGNESIUM ABSORPTION: EXPERIMENT 3

Dependent variable	Equation	R^2	SD	PR>F
Absorption, mMg/d	$Y = -136 + .459x^a$	.88	213	.0001
Apparent absorption, %	$Y = 36.3 + .001x^a$	.05	7.7	.3168
Urinary excretion (7-10d), mg/d	$Y = 97.4 + .308x^a$	.81	187	.0001
Fecal excretion, mg/d	$Y = 136 + .541x^a$	.91	212	.0001
Retention, mg/d	$Y = -233 + .152x^a$	.39	239	.0026
Retention, % intake	$Y = .460 - .002x^a$	.07	8.4	.2313
Urinary excretion (7-10d)m mg/d	$Y = 245 + .619x^a$	.79	197	.0001
Fecal excretion, mg/d	$Y = 594 + .916x^b$	.63	434	.001
Retention, mg/d	$Y = -245 + .380x^b$	.58	198	.001
Retention, % intake	$Y = -1.56 + .006x^b$	.20	7.81	.0404

[a]Where Y equals the dependent variable and x equals Mg intake from sulfate, mg/d

[b]Where Y equals the dependent variable and x equals absorbed Mg from sulfate, mg/d.

TABLE 14. R-SQUARE VALUES FOR REGRESSION MODELS OF URINARY EXCRETION BY DAY AND MAGNESIUM BALANCE TRAITS: EXPERIMENT 3

	R-square			
Variables in model	Absorption mg/d	Apparent Absorption %	Retention mg/d	Retention % intake
Day 1	.338	.377	.000	.006
Day 2	.519	.528	.002	.024
Day 3	.537	.513	.004	.035
Day 4	.488	.493	.014	.058
Day 5	.644	.544	.000	.009
Day 6	.564	.512	.003	.027
Day 1,2	.519	.531	.006	.031
Day 2,3	.540	.531	.006	.044
Day 3,4	.541	.514	.064	.103
Day 4,5	.668	.545	.161	.204
Day 5,6	.663	.544	.108	.109
Day 1,2,3	.540	.533	.012	.053
Day 2,3,4	.543	.533	.064	.106
Day 3,4,5	.670	.547	.164	.205
Day 4,5,6	.673	.546	.182	.215
Day 1,2,3,4	.543	.534	.075	.123
Day 2,3,4,5	.705	.549	.200	.223
Day 3,4,5,6	.675	.548	.186	.217

TABLE 15. RELATIVE BIOAVAILABILITY OF MAGNESIUM SOURCES CALCULATED BY DIFFERENT METHODS: EXPERIMENT 3[a]

Method of Calculation	Sulfate	Magnesite
Absorption mean[b]	100	81.5
Absorption mean[c]	100	59.3
Absorption (Slope Ratio)[d]	100	50.5
Urinary excretion mean (7-10d)[b]	100	54.2
Urinary excretion mean (7-10d)[c]	100	33.0
Urinary excretion mean D4,5[b]	100	51.0
Urinary excretion mean D4,5[c]	100	31.0
Urinary excretion (mean-control) D4,5	100	<0
Relative solubility, %		
H_2O	100	<1
.4% HCl	100	89.4
2% citric acid	100	86.7
Neutral ammonium citrate	100	85.8

[a]Basal diet contained 1275 mg/kg Mg (as-fed basis).

[b]Based on means of groups fed 1000 mg/kg Mg.

[c]Based on means of groups fed 3000 mg/kg Mg.

[d]Based upon slope ratio calculated from 0, 1000, 2000 and 3000 mg/kg added as Mg sulfate and 0, 1000 and 3000 mg/kg Mg as Magnesite.

TABLE 16. EFFECT OF MAGNESIUM SOURCE ON DAILY URINARY MAGNESIUM EXCRETION BY SHEEP: EXPERIMENT 3[a]

	Supplemental Mg, mg/kg					
	Control	Sulfate			Magnesite	
Time, d[b]	0	1000	2000	3000	1000	3000
1	389±97.3	732± 61.8	791± 99.3	746±121.1	412±36.9	391±26.1
2	496±47.6	773± 69.2	1253±124.9	1115± 97.7	430±32.1	380±26.9
3	490±46.0	923±119.2	1319± 96.8	1277± 75.4	432±30.0	403±37.0
4	470±47.4	908±103.4	1290±133.6	1206± 69.8	436±44.1	374±50.5
5	490±35.0	788± 62.9	1423±411.5	1334± 77.0	436±37.9	407±37.8
6	506±40.4	845± 84.7	1492± 62.8	1410± 91.0	471±40.9	353±45.6
7	464±40.4	852± 90.2	1325±136.3	1205± 95.5	407±77.9	450±55.9
8	491±52.2	812±138.7	1512± 62.8	1349± 51.9	487±40.7	467±53.4
9	429±60.8	812± 57.7	1415± 48.7	1338± 74.9	443±47.2	413±36.9
10	459±55.4	827± 90.6	1168±267.3	1335± 55.5	472±60.8	392±44.6

[a]Each value represents the mean ± standard error of six sheep.

[b]Time (days) after feeding the experimental diets.

[c]Control diet contained 1275 mg/kg Mg (as-fed basis).

CHAPTER V
BIOLOGICAL AVAILABILITY OF MAGNESIUM FROM MAGNESIUM OXIDES FED AT HIGH DIETARY LEVELS TO SHEEP: EXPERIMENTS 4 AND 5

Introduction

The essentiality of Mg in animal nutrition and manifestations of Mg deficiency are well documented (Underwood, 1981). Under many conditions supplemental Mg is required to ensure adequate intake to prevent hypomagnesemia. Because the effectiveness of Mg supplements depends on the utilization of the element by the animal, careful consideration of the biological availability of the supplements is required.

Several methods and criteria have been used to determine the bioavailability of Mg sources and results have shown that there was considerable variation in the availability of Mg in different Mg salts (Chicco et al., 1972; Ammerman, et al., 1972; Storry and Rook, 1963; Gerken and Fontenot, 1967). More recent experiments have shown that even within the same Mg salts, availability can vary. Bouwman (1978) compared the relative Mg availability of several feed grade Mg oxides and found considerable variation among them. Wilson (1981) demonstrated that dietary availability of calcined magnesite was correlated with temperature used in the calcination process. Jensen et al. (1986) reported that calcination of Mg oxides caused sintering of Mg crystals at high temperatures which resulted in decreased reactivity. There is also considerable evidence that the availability of Mg oxides is dependent upon particle size. Jesse et al. (1981) demonstrated

that increased mesh size (smaller particles) produced a more rapid excretion of Mg in urine after oral administration and Jensen et al. (1986) found increased reactivity with decreasing particle size. All of the above factors contribute to the "ultimate" availability for different Mg oxides.

Results of previous experiments indicated that a new bioassay technique using urinary Mg excretions of sheep fed Mg in excess of the requirement could be useful in determining Mg bioavailability of Mg oxides. In the following experiments the relative bioavailability of Mg from several feed grade oxides was determined by using both balance methods and the new bioassay technique.

Materials and Methods

Two Mg balance trials were conducted with thirty-five crossbred wethers averaging 43.0 kg initial weight. Treatments were assigned randomly to sheep housed in individual metabolism crates designed for separate collection of urine and feces. The basal diet was a conventional corn-soybean meal-cottonseed hulls diet (Table 3) which was formulated according to NRC (1985) recommendations. Dietary treatments consisted of the basal diet supplemented with either 0, 700, 1400 or 2100 mg/kg Mg as a reagent grade $MgSO_4$ or 1400 mg/kg Mg from either oxide E, F or G (Experiment 4) or 1400 mg/kg from either oxide G, H or I (Experiment 5). Feed intake was restricted to 1000 g daily and tap water was available ad libitum. Sheep were fed the basal diet for 10 days prior to the 10-day experimental period. Urine collection started 2 days prior to the experimental period and

continued daily throughout the experiment. Fecal collections were made only during the last 4 days of the experimental period. Collection and analytical procedures were similar to those described in Experiments 1 and 2.

Data were analyzed by the general linear models procedure (Proc GLM) of the SAS System (SAS, 1985). Treatment means were separated by Duncan's multiple range test. Regression analysis was performed for different parameters to determine slope-ratios among different Mg sources. Daily urinary excretion time trends were analyzed using repeated measures in time analysis with the Helmert option of Proc GLM to determine when the rate of increase occurance in urinary excretion. All possible subsets regression was performed to determine relationships between Mg absorption or retention and daily urinary Mg excretion in order to select for the best combination of days to make urinary collections.

Results

Characterization of the Mg sources used in Experiment 4 is found in Table 17. Oxide E was a calcined magnesite with very minor $Mg(OH)_2$ and $Ca(OH)_2$ and traces of olivine and quartz. Oxide-F was a calcined magnesite with minor monticellite and oxide-G was derived from brine. Chemical constituents other than MgO determined by x-ray spectroscopy were low for oxide E and G, but not for oxide-F. The analyzed Mg concentrations were 14.7% for sulfate and 48.7, 45.3 and 55.6% for oxides E, F and G, respectively. Relative solubilities for the different Mg oxides used in Experiment 4 are also found in Table 17. Oxide E was the

most soluble source and did not show much variation in solubility when tested in the different solvents. However, in .4% HCl oxide G was more soluble than oxide F (83.9% vs 79.5%) but, in 2% citric acid, oxide F was more soluble than oxide G (81.2% vs 79.6%). The solubility for oxides F and G was lower in neutral ammonium citrate while that for oxide E remained similar.

The Mg intake of sheep (Table 19) was not different among the sulfate, and Mg oxides at equal levels of supplementation. Increasing Mg supplementation from 0 to 2100 mg/kg Mg increased Mg intake from 1184 to 3352 mg/kg Mg. Fecal Mg excretions (Table 19) increased linearly (R^2 = .96) with increasing levels of Mg sulfate supplementation but was not different (P > .05) for sheep fed either the Mg sulfate or Mg oxides at equal levels of supplementation. Increases in Mg absorption were linear (R^2 = .84) as dietary Mg sulfate supplementation increased but were not different (P > .05) among the different Mg oxides and sulfate. Expressing total Mg absorption as a percentage of the intake (apparent absorption) to compare the different treatment at equal intakes resulted in no significant differences. However, sheep receiving 700 Mg/kg Mg as sulfate had a higher percentage apparent absorption when compared to all other treatment groups except for sheep fed 1400 Mg/kg Mg as oxide E and 2100 mg/kg as sulfate. No differences (P > .05) were observed in urinary Mg excretion when treatments were compared at the same supplementary level. Increases in urinary Mg excretion showed a linear trend (R^2 = .75) as Mg sulfate supplementation increased. The net Mg retention, as well as retention as a percentage of intake, were

insensitive to dietary supplementation. Regression analysis performed on these parameters indicated no significant linear response. Final serum Mg concentrations of sheep fed the control diet were lower ($P < .05$) when compared to sheep fed 2100 Mg/kg Mg as sulfate and sheep fed 1400 Mg/kg Mg as oxide G. All treatments had greater ($P < .05$) final serum Mg concentrations when compared to the initial values. The change in serum Mg concentration was greatest for sheep fed 2100 Mg/kg Mg as sulfate which was higher ($P < .25$) when compared to the control group.

In the regression analysis of various Mg balance parameters (Table 20), the best fit was obtained with fecal Mg excretion, followed by absorption and urinary Mg excretion when Mg intake was used as the independent variable. When Mg absorption was used as the independent variable, the best fit to a linear model resulted when urinary Mg excretion was the dependent variable followed by fecal Mg excretion. The R-square values obtained with net Mg retention and retention as a percentage of intake were too low to conclude significant linearity for these measurements.

To use Mg absorption as the criterion for determination of Mg availability, regressions were performed where Mg absorption was the response variable, and Mg intake as the independent variable. Slopes obtained were .304, .232, .222 and .247 for sulfate, oxide E, F and G, respectively. Magnesium sulfate was equated to 100% and the respective Mg availabilities for oxide E, F and G were 76.6, 73.3 and 81.5% when 0 and 1400 mg/kg for each source were used in the calculation.

Regression of daily urinary Mg excretion on Mg absorption or retention (Table 21) was used to determine which day or days were most sensitive for comparison of relative availability. The largest R-square value was obtained with day 5 when only one variable (day) was used in the model. When 2 days were used in the model, days 4, 5 gave the best fit. Little improvement in the R^2 value was observed when additional days were added in the model and improvement which was observed was most likely due to increasing the number of variables. When apparent absorption, Mg retention and retention as a percentage of intake were used as independent variables (Table 22) in the regressions with daily urinary Mg excretion (dependent variable), the R^2 values obtained were too low to take these criteria into consideration for the selection of days to determine Mg availability. When the mean urinary Mg concentration on day 4,5 was used as the criterion (Table 22), the relative bioavailability for sulfate, and oxides E, F and G was 100, 77.9, 77.5 and 86.3% , respectively. When the increase of the mean urinary Mg concentrations of day 4, 5 over the control group was used, i.e. the relative increase in Mg excretion due to supplementation, the following availabilities were calculated for sulfate, and oxides E, F and G: 100, 64.4, 63.7 and 77.9 respectively.

Table 23 shows the increase of urinary Mg concentration over time for each treatment. To determine the time interval for urinary Mg excretions to plateau, analysis was made using the Helmert option of the GLM (SAS) procedure. The mean urinary Mg concentration of a specific day was compared with the mean Mg

concentration of the remaining days; Urinary Mg concentrations for sheep fed 1400 or 2100 mg/kg Mg as sulfate and oxide E, F and G plateaued ($P > .10$) after day 2, and sheep fed 700 mg/kg Mg as sulfate had urinary Mg concentrations which plateaued after day 1. Sheep fed the control diet had urinary Mg excretions which did not differ from the pre-experimental period.

The physical properties of the Mg oxides used in Experiment 5 are listed in Table 24. Oxides H and I were both calcined magnesites while source G was derived from brine. Particle size distribution varied among the Mg oxide sources with oxide I having the highest percentage of smaller particles. Oxide G had the most homogeneous distribution in particle size.

Analyzed Mg concentrations were 14.7, 55.6, 53.7 and 47.0% for Mg sulfate and oxides G, H, and I, respectively. Analysis of chemical constituents for each of the sources showed that oxide G had the lowest concentration of impurities. Oxide H had relatively large amounts of SiO_2, $Fe2O3$, Al_2O_3, CaO, SO_3 and CL^3 When compared to oxides G and I. Relative solubilities (Table 24) for oxide H and G were variable when tested in different solvents while solubility for oxide I remained quite constant when tested in these different solvents. Oxide I had the greatest solubility in all solvents of all the oxides. When solubility was tested in .4% HCl and neutral ammonium citrate, oxide H was more soluble than oxide G but when solubility was tested in 2% citric acid, oxide G was more soluble than oxide H.

Magnesium intake per sheep (Table 26) increased from 1277 mg/day to 3377 mg/day as Mg supplementation increased from 0 to

2100 mg/kg. At equal levels of supplementation, intake was not different ($P > .05$) among the sulfate and the Mg oxides. Fecal excretion (Table 26) increased ($P < .05$) as dietary supplementation increased. Increases in fecal Mg excretion were linear ($R^2 = .84$) as shown by the regression analysis between fecal Mg excretion and Mg intake for sheep fed Mg sulfate (Table 27). Fecal Mg excretion did not differ ($P > .05$) among sources fed at 1400 mg/kg. Absorption (Table 26) increased with dietary supplementation. Increases in Mg absorption were linear ($R^2 = .93$) as intake of Mg sulfate (Table 27) increased. When absorption was compared among the different oxides and a comparable level of sulfate, no differences ($P > .05$) were observed. Apparent absorption was insensitive to dietary Mg intake and was not different ($P > .10$) for the different treatment groups. Increases in urinary Mg excretion (Table 26) were linear ($R^2 = .89$) as indicated by the regression analysis on increasing levels of dietary sulfate (Table 27). Urinary Mg excretion of sheep fed 1400 mg/kg Mg as sulfate was greater ($P < .01$) when compared to urinary Mg excretion of sheep fed any of the Mg oxides. Urinary Mg excretions were not different ($P > .05$) among the different oxides. Retention of Mg (Table 26) increased ($P < .01$) as Mg intake increased. Regression analysis between Mg retention and Mg intake (Table 27) indicated a linear increase ($R^2 = .73$) as sulfate supplementation increased. At an equal level of supplementation, Mg retention for sheep fed Mg sulfate was not different ($P > .05$) from sheep fed oxide H and I but was lower ($P < .01$) compared to sheep fed oxide G. When

retention was expressed as a percentage of intake, no differences ($P > .05$) were observed among the different treatment groups. Final serum Mg concentration did not differ ($P > .05$) among treatments, however, all sheep fed the Mg sources had greater ($P < .05$) final serum Mg concentrations when compared with initial values (Table 26).

Regression analysis (Table 27) indicated significant ($P < .0001$) relationships between Mg intake and absorption, urinary excretion, fecal excretion and Mg retention. In these regressions, where Mg intake was the independent variable, the highest R-square value was obtained using Mg absorption as the dependent variable. When regressions were performed using Mg absorption as the independent variable, urinary Mg excretion gave the highest R-square value ($R^2 = .88$) indicating a strong relationship between these parameters. All other traits (fecal net excretion, Mg retention and retention, % I) used in the regressions where Mg absorption was the independent variable were significant ($P < .001$). Magnesium absorption as the dependent variable was regressed on Mg intake for each source and the following slopes were derived for sulfate and oxides G, H and I, .569, .534, .472, and .505. Equating the sulfate to 100% gave the following percentages of relative availability: sulfate (100%), oxide G (93%), oxide H (83%) and oxide I (89%).

To determine the relationship between daily urinary Mg excretion and several balance traits (absorption, apparent absorption, retention, retention % I) regressions were performed (Table 28). The R-square values were used as criteria to select

for the best day or combination of days which were most indicative of bioavailability. The best R-square values were obtained by the regression using Mg absorption as dependent variable. The R-square values obtained by using Mg retention were consistently higher than those of the apparent absorption and retention % intake.

Irrespective of the dependent variable used in the regressions, day 5 had the best fit when a single day's excretion was used in the model. Using two consequtive collection days in the model, the best R-square value was found for days 4,5. When 3 days of urinary collection were used as dependent variable in the model, little improvement was made and the best combinations appeared to be either day 4, 5 and 6 or day 3, 4 and 5. Using the mean urinary excretions on days 4, 5 to calculate the Mg availability (Table 29) the following availabilities were calculated for sulfate and oxides G, H and I; 100, 85.3, 77.6 and 81.8. When the increase in urinary Mg over the control group (mean 4,5 - control) was used the relative availabilities were: 100, 73.8, 59.9 and 67.3 for sulfate and oxides G, H and I, respectively.

The Helmert option of the GLM procedure was used on daily urinary excretions during the 10-d experimental period (Table 30) to determine for each treatment at which day after feeding the treatment diets urinary Mg excretion plateaued. For sheep fed the control diet, urinary Mg excretion during the experimental period did not differ from that prior to the experimental period (pre). For oxide G urinary Mg excretions plateaued after day 1

while for oxide H and I this happened on day 2. For sheep fed diets supplemented with 1400 mg/kg Mg as sulfate urinary Mg concentrations plateaued after day 3.

Discussion

With the present procedure, variation in feed intake (hence Mg intake) will influence the accuracy of estimates of Mg availability. In both Experiments the total Mg intake among the comparable treatment groups was not different and therefore no correction of intake was necessary. As was suggested in Experiment 3, dietary concentrations up to 2100 mg/kg were used instead of 3000 mg/kg. In both Experiments (4 and 5) no feed refusal or problems with diarrhea were observed for sheep consuming diets supplemented with 2100 mg/kg Mg as sulfate. This indicated that levels up to 2100 mg/kg are safe and can be used without problems in future Mg bioassays.

As already observed in earlier experiments, increases in absorption were linear as level of Mg supplementation increased. These and present observations therefore indicated that at these supplementary levels no homeostatic mechanism is in control of absorption which is of particular importance if estimates of relative availability are based on absorption. Similar findings were also reported by several other workers (Chicco et al., 1973; Araujo, 1976; and Bouwman, 1978) who suggested that the increase in dietary intake would ultimately lead to an increase in urinary Mg excretions, reflecting an increase in gut absorption of Mg.

When relative Mg availability calculations were based on either absorption or urinary excretion, results obtained with

solubilities of the different oxide sources used in Experiments 4 and 5 did not correlate well with the values on relative availabilities for each of these sources (Tables 22 and 29). This however is not in agreement with the results obtained in Experiments 1 and 2 where solubilities in 2% citric acid and neutral ammonium citrate were indicative of the relative availabilities of the oxide sources used.

The positive relationship observed between Mg absorption and urinary Mg excretion in these experiments again demonstrates the validity of using urinary excretion as an indicator of the amount of Mg absorbed in excess of the requirement. Fecal Mg excretion also showed a strong relationship with Mg intake which indicates the possible use of this parameter as an indicator of the unabsorbed Mg. Shockey et al. (1984), who used measurements of fecal Mg excretions, found good applications of this method to determine relative availability. In the present experiments however, this method was not included.

Net retention of Mg, which is also frequently used to estimate bioavailability, had a poorer fit to the linear model when compared to Mg absorption. In Experiment 5 however, considerable improvement in fit for the linear model was observed for Mg retention however, the R-square value was still lower than for Mg absorption. The lower response and the poorer fit in regressions therefore makes Mg retention a less sensitive measure for estimating Mg availability. These conclusions are in agreement with the results of other workers (Thompson, 1965; Chicco et al., 1972; Bouwman, 1978) who found this measurement

was unacceptable for use in Mg availability determinations.

In both experiments, final serum Mg concentrations were increased over the initial values. This was also observed for the control group which indicates that the control diet supplied more Mg than diets fed prior to the experimental period. Increases in dietary supplementation caused no significant increases in serum Mg concentrations. This indicates that serum Mg concentrations are insensitive when Mg is absorbed in excess of the requirements.

Since Mg absorption was the trait that was most sensitive to dietary Mg supplementation, measurements of relative bioavailability of the various Mg oxides in Experiments 4 and 5 were based on this criterion. The mean absorption for oxide G in Experiments 4 and 5 resulted in availability estimates of 89 and 95%, respectively, compared with sulfate. This indicates that various uncontrollable conditions can effect measurements on availability and therefore less emphasis must be placed on absolute values and more attention must be given to relative rankings among the sources. Bioavailability estimates for oxide G based on urinary excretion means on days 4,5 were 86 and 85% of sulfate, respectively. Thus, it appeared in these experiments that there was less variability associated with urinary excretion of Mg than absorption. Some of the variation may be explained by environmental conditions during different seasons of the year. Oxide A from Experiment 2 was similar to oxide G in composition, but it had a greater percentage of smaller particles than oxide G. Based on means of absorption and urinary Mg excretion on day

4,5, availability of oxide A was 88 and 80% of sulfate, respectively. Thus, estimates of bioavailability were reasonably consistent.

The good fit of the regressions between absorption and urinary excretions demonstrate that a major portion of Mg absorbed in excess of the requirement will be excreted via the urine. Thus, an animal receiving an oral load of a readily available Mg source would exhibit a transitory increase in urinary Mg excretion, whereas a less available Mg source would produce a smaller increase. Using this relationship, selections of daily urinary excretions were made which are highly indicative for the Mg availability. From the results of Experiments 4 and 5 it appeared that collections from day 4 and day 5 are most likely to give the best indications. This also agrees with the conclusion that valuable comparisons between sources can only be made at intervals were urinary Mg excretions remain stable, i.e. after plateaus. In both Experiments urinary excretions for all the comparable treatment groups plateaued no later than day 4. There were no significant differences in bioavailability estimates of sulfate and oxides in Experiments 4 and 5; however, none of the oxide, was valued at 100% of sulfate. The oxide of brine origin was as well utilized as those from calcined magnesite. Although not different statistically, these estimates may be used in conjunction with economical considerations in choosing a supplemental Mg source.

Summary

Two experiments were conducted to estimate the biological availability of Mg from Mg oxides fed at high dietary concentrations to sheep. In each of two experiments thirty-five crossbred wethers, averaging 45 kg initially, were housed in individual metabolism cages designed for separate collections of urine and feces. During a 10-day adjustment period which preceeded the experimental period, sheep were fed the basal diet. At initiation of the experimental period, blood samples were taken and sheep were assigned randomly to one of seven dietary treatments (five sheep/treatment) for a period of 10 days. Dietary treatments were the basal diet (corn-soybean meal-cottonseed hulls) supplemented with either 0, 700, 1400 or 2100 mg/kg Mg as reagent Mg sulfate or 1400 mg/kg Mg from either oxide E, F or G (Experiment 4) or oxide G, H or I (Experiment 5). Increases in dietary supplementation resulted in linear increases ($P < .01$) in Mg absorption, fecal Mg excretion, and urinary Mg excretion. Urinary Mg excretions were highly related with Mg absorption which justified the use of this parameter for measurements of relative Mg availability. In both Experiments no differences were observed for Mg absorption when dietary treatments were compared at equal levels of supplementation. Results from both experiments also indicated that urinary collections made at day 4,5 were most indicative of Mg availability when absorption was used as the criterion. When the slope ratios were used to express Mg availability, values for sulfate and oxides E, F and G were 100, 76.6, 73.3 and 81.5%,

respectively. The mean urinary excretions from day 4,5 resulted in relative availability estimates of 100, 77.9, 77.5 and 86.3% for sulfate and oxides E, F and G, respectively. In Experiment 5, the relative availabilities for sulfate and oxides G, H and I were 100, 86.9, 76.9 and 82.2% when the slope ratio of absorption was used. Calculating Mg availabilities using mean urinary excretions of day 4,5 resulted in relative availabilities of 100, 85.3, 77.6 and 81.8% for sulfate and oxides G, H and I, respectively. Based upon these results it appeared that values of Mg availability calculated using mean urinary excretions from day 4,5 were similar to relative availabilities calculated using Mg absorption. It may therefore be concluded that mean urinary Mg concentrations are useful as indicators of Mg availability when Mg is supplemented in excess of requirement.

TABLE 17. PHYSICAL AND CHEMICAL CHARACTERIZATION OF MAGNESIUM SOURCES FED TO SHEEP: EXPERIMENT 4

Item	Sulfate (RG)[a]	Oxide (FG)[a]		
		E	F	G
Origin	Unknown	Calcined magnesite	Calcined magnesite	Brine
Composition[b]	$MgSO_4$	MgO; very minor $Mg(OH)_2$ and $Ca(OH)_2$ traces of olivine and quartz	MgO; minor Monticellite $CCaMgSiO_4$	MgO
Particle size, %				
+20	.1	.9	NA	.2
+100	90.4	30.7	NA	98.9
+200	9.2	20.3	NA	.5
-200	.3	48.1	NA	.7
Loss on iginition, %	NA[c]	5.78	.20	.15
Chemical constituents, %[d]				
MgO	NA	88.57	84.66	98.59
SiO_2	NA	.92	5.04	.40
Fe_2O_3	NA	.54	3.02	.12
Al_2O_3	NA	.29	1.63	.07

TABLE 17. Continued

Item	Sulfate	Oxide (FG)		
		E	F	G
Cr_2O_3	NA	.00	.00	.00
CaO	NA	3.83	5.18	.67
SO_3	NA	.07	.027	.00
Mg[e]	14.7	48.7	45.3	55.6
Relative solubility, %				
H_2O	99.6	.88	.13	.42
.4% HCl	100	96.1	79.5	83.9
2% Citric Acid	100	95.5	81.2	79.6
Neutr. amm. citr.	100	93.4	27.9	41.3

[a]RG = reagent grade, FG = feed grade.

[b]By x-ray diffraction analysis (International Minerals and Chemical Corp., Mundelein, IL).

[c]NA = not analyzed.

[d]By x-ray spectroscopy (National Magnesia Chemicals, Moss Landing, CA).

[e]Analyzed percentage on the as-fed source following 4 h refluxing with 1:1 (v:v) $HCl-HNO_3$.

TABLE 18. COMPOSITION OF THE BASAL DIET: EXPERIMENT 4

Item	%[a]
Corn, ground	57.45
Cottonseed hulls	21.00
Soybean meal, dehulled	12.00
Alfalfa meal	3.00
Salt, trace-mineralized[b]	.50
Vitamins[c]	+
Ethyoxyquin	.0125
Limestone, ground	.55
Corn oil[d]	3.00
Cornstarch[e]	2.50

[a]As-fed basis. Contained 88.9% dry matter.

[b]Contained the following (%): 93 NaCl; .35 Zn; .28Mn; .175 Fe; .035 Cu; .007 I; .007 Co.

[c]Vitamins added per kg of diet: 2,200 IU vitamin A palmitate; 440 IU vitamin D_3.

[d]Ethoxyquin was added to the diet at .0125% as an antioxidant (Monsanto Chemical Co., St. Louis, MO).

[e]Magnesium sources were added at the expense of cornstarch.

TABLE 19. EFFECT OF MAGNESIUM SOURCE ON MAGNESIUM BALANCE AND SERUM MAGNESIUM CONCENTRATION IN SHEEP: EXPERIMENT 4[a]

	Supplemental Mg, mg/kg							
	Control	Sulfate			Oxide E	Oxide F	Oxide G	
Magnesium	0 (5)[b]	700 (4)	1,400 (5)	2,100 (5)	1,400 (5)	1,400 (4)	1,400 (4)	SEM
Intake, mg/d	1,184[g]	1,935[f]	2,652[d]	3,352[c]	2,236[cf]	2,493[de]	2,652[d]	52.4
Fecal excretion, mg/d	823[f]	1,146[cf]	1,849[cd]	2,211[c]	1,512[de]	1,794[cd]	1,935[cd]	57.8
Absorption, mg/d	361[e]	785[d]	803[d]	1140[c]	724[d]	660[d]	717[d]	22.3
Apparent absorption, %	30.2[d]	40.6[c]	30.3[d]	34.0[cd]	34.6[cd]	27.4[d]	27.0[d]	1.3
Urinary excretion, mg/d	421[e]	797[d]	887[b]	1,080[c]	753[d]	778[d]	809[d]	27.0
Net retention, mg/d	-59.6[c]	-30.2[c]	-84.3[c]	59.4[c]	-29.3[c]	-90.3[c]	-96.2[c]	27.12
Retention, % intake	-4.99[c]	1.5[c]	-3.18[c]	1.8[c]	0.56[c]	-3.4[c]	-3.6[c]	1.2
Final serum, mg/dl	2.18[d]	2.36[cd]	2.37[cd]	2.56[c]	2.36[cd]	2.42[cd]	2.49[c]	.03
Change serum, mg/dl	-.006[d]	.18[cd]	.17[cd]	.29[c]	.19[cd]	.14[cd]	.15[cd]	.03

[a]Control diet contained 1252 mg/kg Mg (as-fed basis). Based on fecal and urinary collections from d7 through d10.

[b]Number in parentheses represents the number of sheep/treatment.

c,d,e,f,gMeans in the same row without a common superscript differ ($P < .05$).

TABLE 20. LINEAR REGRESSION OF VARIOUS MAGNESIUM BALANCE TRAITS ON MAGNESIUM INTAKE AND MAGNESIUM ABSORPTION IN SHEEP SUPPLEMENTED WITH MAGNESIUM SULFATE: EXPERIMENT 4

Dependent variable	Equation	R^2	SD	PR>F
Absorption, mg/d	$Y = 10.1 + .331x^a$	.84	125	.0001
Apparent absorption, %	$Y = 32.2 + .0005x^a$	.006	6.06	.7561
Urinary excretion (D 7-10), mg/d	$Y = 136 + .290x^a$	.75	141	.0001
Fecal excretion, mg/d	$Y = -10.1 + .669x^a$	.96	125	.0001
Retention, mg/d	$Y = -123 + .041x^a$	.11	103	.1677
Retention, % intake	$Y = -7.98 + .003x^a$	.20	4.61	.0582
Urinary excretion (D 7-10), mg/d	$Y = 130 + .869x^b$	.88	101	.0001
Fecal excretion mg/d	$Y = 336 + 1.54x^b$	.66	347	.0001
Retention, mg/d	$Y = -129 + .131x^b$	.14	101	.1105
Retention, % intake	$Y = -7.69 + .007x^b$	.20	4.58	.0521

[a]Where Y equals the dependent variable and x equals Mg intake from sulfate, mg/d.

[b]Where Y equals the dependent variable and x equals Mg absorbed from sulfate, mg/d.

TABLE 21. R-SQUARE VALUES FOR REGRESSION MODELS OF URINARY EXCRETION BY DAY AND MAGNESIUM BALANCE TRAITS: EXPERIMENT 4[a]

	R-square			
Variables in model	Absorption, mg/d	Apparent absorption, %	Retention, mg/d	Retention, % intake
Day 1	.089	.044	.028	.013
Day 2	.270	.063	.016	.031
Day 3	.579	.055	.003	.006
Day 4	.632	.180	.064	.082
Day 5	.711	.172	.052	.080
Day 1,2	.283	.065	.154	.153
Day 2,3	.583	.069	.019	.037
Day 3,4	.671	.217	.135	.153
Day 4,5	.724	.180	.071	.083
Day 1,2,3	.704	.069	.162	.157
Day 2,3,4	.675	.229	.149	.181
Day 3,4,5	.758	.217	.139	.154
Day 4,5,6	.727	.188	.100	.094
Day 1,2,3,4	.764	.236	.250	.260

[a]Absorption and retention calculated from samples collected on D 7 through 10. Represents data from 35 sheep.

TABLE 22. RELATIVE BIOAVAILABILITY OF MAGNESIUM SOURCES CALCULATED BY DIFFERENT METHODS: EXPERIMENT 4

Method of calculation	Sulfate	Oxide E	Oxide F	Oxide G
Absorption mean[b]	100	90.2	82.2	89.3
Absorption (slope ratio)[c]	100	76.6	73.3	81.5
Absorption (slope ratio)[d]	100	70.1	67.1	74.6
Urinary excretion mean D 7-10[b]	100	84.9	87.7	91.2
Urinary excretion mean D 4,5[b]	100	77.9	77.5	86.3
Urinary excretion (mean - pre) D 4,5[b]	100	66.2	89.3	67.5
Urinary excretion (mean - control) Day 4,5[b]	100	64.4	63.7	77.9
Relative solubility, %				
H2O	100	<1	<1	<1
.4% HCl	100	96.1	79.5	83.9
2% Citric acid	100	95.9	81.2	79.6
Neutral ammonium citrate	100	93.4	27.9	41.3

[a]Basal practical-type diet contained 1252 mg/kg Mg (as-fed basis).

[b]Based on means of groups fed 1,400 mg/kg added Mg from from all sources.

[c]Based on slope ratio calculated from 0 and 1,400 mg/kg added Mg from all sources.

[d]Based on slope ratio calculated from 0, 700, 1,400 and 2,100 mg/kg added Mg from sulfate and 0 and 1,400 mg/kg from oxides.

TABLE 23. EFFECT OF MAGNESIUM SOURCE ON DAILY URINARY MAGNESIUM EXCRETION BY SHEEP: EXPERIMENT 4[a]

	Supplemental Mg, mg/kg						
	Control	Sulfate			Oxide E	Oxide F	Oxide G
Time, d[b]	0[c]	700	1,400	2,000	1,400	1,400	1,400
Pre	276 ± 84.7[d]	317 ± 100.8	330 ± 59.5	201 ± 76.5	330 ± 36.0	182 ± 69.0	402 ± 97
1	396 ± 46.8	634 ± 122.5[d]	662 ± 120.2	456 ± 95.9	544 ± 92.8	579 ± 79.1	605 ± 70
2	333 ± 58.8	730 ± 155.2	729 ± 197.8[d]	755 ± 149.5[d]	607 ± 81.6[d]	744 ± 165.1[d]	859 ± 87
3	417 ± 95.8	793 ± 106.9	904 ± 131.7	951 ± 74.7	797 ± 103.8	779 ± 67.4	835 ± 60
4	371 ± 73.3	695 ± 58.0	965 ± 169.2	969 ± 56.1	739 ± 87.0	734 ± 94.2	817 ± 94
5	354 ± 63.5	631 ± 55.1	944 ± 131.3	992 ± 65.2	748 ± 66.9	745 ± 81.5	830 ± 97
6	361 ± 66.4	695 ± 56.8	945 ± 149.6	995 ± 58.8	728 ± 91.4	721 ± 75.0	814 ± 86
7	361 ± 93.4	809 ± 73.3	862 ± 63.3	1,074 ± 107.1	691 ± 75.6	734 ± 72.8	868 ± 53
8	434 ± 53.3	801 ± 73.8	924 ± 107.5	1,148 ± 47.2	772 ± 112.7	731 ± 65.5	809 ± 105
9	495 ± 85.4	829 ± 73.4	931 ± 86.1	1,178 ± 31.2	818 ± 154.5	862 ± 56.5	815 ± 42
10	370 ± 56.6	749 ± 70.1	927 ± 75.2	1,148 ± 53.7	679 ± 39.1	794 ± 30.8	740 ± 81
Mean D 7-10	421	797	887	1,080	753	778	809

[a]Each value represents the mean ± standard error of five sheep.

[b]Time after feeding experimental diets. Pre represents the mean from the 2 d prior to feeding experimental diets.

[c]Control diet contained 1252 mg/kg Mg (as-fed basis).

[d]Within a dietary treatment, no difference (P > .10) between this day and the mean of the remaining days.

TABLE 24. PHYSICAL AND CHEMICAL CHARACTERIZATION OF MAGNESIUM SOURCES FED TO SHEEP: EXPERIMENT 5

Item	Sulfate (RG)	Oxide (FG)		
		G	H	I
Origin	Unknown	Brine	Calcined Magnesite	Calcined Magnesite
Composition[b]	$MgSO_4$	MgO	MgO; very minor $MgCO_3$, $Mg(OH)_2$	MgO; very minor $CaCO_3$, olivine
Particle size, %				
+20	.1	.2	2.8	6.2
+100	90.4	98.9	81.6	66.1
+200	9.2	.5	4.6	8.6
-200	.3	.7	11.0	19.1
Loss on iginition, %	NA[c]	.15	7.67	.13
Chemical constituents, %[d]				
MgO	NA	98.59	87.0	97.4
SiO_2	NA	.40	1.00	.24
Fe_2O_3	NA	.12	.54	.51
Al_2O_3	NA	.07	.25	.12

TABLE 24. Continued

Item	Sulfate (RG)	Oxide G	Oxide H	Oxide I
Cr_2O_3	NA	.00	.00	.00
CaO	NA	.67	3.51	1.56
SO_3	NA	.00	.03	.00
Mg[e]	14.7	55.6	53.7	47.0
Relative solubility, %				
H_2O	99.6	.42	.34	1.39
.4% HCl	100	83.9	90.0	97.3
2% Citric Acid	100	79.6	68.8	96.8
Neutr. amm. citr.	100	41.3	48.3	93.9

[a]RG = reagent grade, FG = feed grade.

[b]By x-ray diffraction analysis (International Minerals and Chemical Corp., Mundelein, IL).

[c]NA = not analyzed.

[d]By x-ray spectroscopy (National Magnesia Chemicals, Moss Landing, CA).

[e]Analyzed percentage on the as-fed source following 4 h refluxing with 1:1 (v:v) $HCl-HNO_3$.

TABLE 25. COMPOSITION OF THE BASAL DIET: EXPERIMENT 5

Item	%[a]
Corn, ground	57.45
Cottonseed hulls	21.00
Soybean meal, dehulled	12.00
Alfalfa meal	3.00
Salt, trace-mineralized[b]	.50
Vitamins[c]	+
Ethyoxyquin	.0125
Limestone, ground	.55
Corn oil[d]	3.00
Cornstarch[e]	2.50

[a]As-fed basis. Contained 88.9% dry matter.

[b]Contained the following (%): 93 NaCl; .35 Zn; .28 Mn; .175 Fe; .035 Cu; .007 I; .007 Co.

[c]Vitamins added per kg of diet: 2,200 IU vitamin A palmitate; 440 IU vitamin D_3.

[d]Ethoxyquin was added to the diet at .0125% as an antioxidant (Monsanto Chemical Co., St. Louis, MO).

[e]Magnesium sources were added at the expense of cornstarch.

TABLE 26. EFFECT OF MAGNESIUM SOURCE ON MAGNESIUM BALANCE AND SERUM MAGNESIUM CONCENTRATION IN SHEEP; EXPERIMENT 5[a]

	Supplemental Mg, mg/kg							
	Control	Sulfate			Oxide G	Oxide H	Oxide I	
Magnesium	0 (4)[b]	700 (5)	1,400 (4)	2,100 (5)	1,400 (5)	1,400 (5)	1,400 (5)	SEM
Intake, mg/d	1,277[f]	1,803[e]	2,677[d]	3,377[c]	2,613[d]	2,677[d]	2,589[d]	35.2
Fecal excretion, mg/d	674[d]	836[d]	1,277[c]	1,466[c]	1,286[c]	1,412[c]	1,304[c]	41.4
Absorption, mg/d	603[f]	968[e]	1,400[d]	1,930[c]	1,327[d]	1,264[d]	1,285[d]	40.6
Apparent absorption, %	47[e]	53[e]	52[e]	57[e]	51[e]	47[e]	50[e]	1.5
Urinary excretion, mg/d	416[e]	660[d]	960[a]	1,136[a]	620[d]	755[d]	696[d]	29.2
Net retention, mg/d	166[f]	307[ef]	441[ef]	794[e]	706[ed]	509[cde]	589[cde]	39.4
Retention, % intake	13[e]	17[e]	16[e]	24[e]	27[e]	19[e]	23[e]	1.5
Final serum, mg/dl	2.52[e]	2.39[a]	2.44[e]	2.39[e]	2.32[e]	2.29[e]	2.46[e]	.04
Change serum, mg/dl	.33[e]	.15[ed]	.21[ed]	.03[d]	.11[ed]	.12[ed]	.26[ed]	.03

[a]Control diet contained 1277 mg/kg Mg (as-fed basis). Based on fecal and urinary collections from d7 through d10.

[b]Number in parentheses represents the number of sheep/treatment.

[a,d,e,f]Means in the same row without a common superscript differ ($P < .01$).

TABLE 27. LINEAR REGRESSION OF VARIOUS MAGNESIUM BALANCE TRAITS ON MAGNESIUM INTAKE AND MAGNESIUM ABSORPTION IN SHEEP SUPPLEMENTED WITH MAGNESIUM SULFATE: EXPERIMENT 5

Dependent variable	Equation	R^{2a}	SD[b]	PR>F
Absorption, mg/d	$Y = -172 + .614x^a$	.93	150	.0001
Apparent absorption, %	$Y = 43 + .0039x^a$	.22	6.4	.0497
Urinary excretion (D 7-10), mg/d	$Y = 5.2 + .344x^a$	.89	105	.0001
Fecal excretion, mg/d	$Y = 173 + .386x^a$	.84	150	.0001
Retention, mg/d	$Y = -207 + .279x^a$	.73	150	.0001
Retention, % intake	$Y = 8.43 + .004x^a$	.31	5.3	.0168
Urinary excretion (D 7-10), mg/d	$Y = 156 + .523x^a$	.88	107	.0001
Fecal excretion mg/d	$Y = 427 + .512x^b$	.60	235	.0002
Retention, mg/d	$Y = -156 + .477x^b$	.86	107	.0001
Retention, % intake	$Y = 7.8 + .008x^b$	.49	4.6	.0011

[a]Where Y equals the dependent variable and x equals Mg intake from sulfate, mg/d.

[b]Where Y equals the dependent variable and x equals Mg absorbed from sulfate, mg/d

TABLE 28. R-SQUARE VALUES FOR REGRESSION MODELS OF URINARY EXCRETION BY DAY AND MAGNESIUM BALANCE TRAITS: EXPERIMENT 5[a]

	R-square			
Variables in model	Absorption, mg/d	Apparent absorption, %	Retention, mg/d	Retention, % intake
Day 1	.416	.125	.255	.096
Day 2	.564	.216	.255	.066
Day 3	.589	.195	.186	.012
Day 4	.650	.313	.289	.076
Day 5	.778	.412	.509	.220
Day 1,2	.591	.218	.300	.099
Day 2,3	.660	.236	.262	.081
Day 3,4	.678	.316	.291	.128
Day 4,5	.784	.412	.537	.293
Day 1,2,3	.661	.237	.300	.149
Day 2,3,4	.696	.317	.308	.139
Day 3,4,5	.789	.434	.572	.432
Day 4,5,6	.788	.417	.559	.357
Day 1,2,3,4	.697	.332	.326	.176
Day 2,3,4,5	.791	.463	.602	.477
Day 3,4,5,6	.792	.439	.596	.505

[a]Absorption and retention calculated from samples collected on D 7 through 10. Represents data from 35 sheep.

TABLE 29. RELATIVE BIOAVAILABILITY OF MAGNESIUM SOURCES CALCULATED BY DIFFERENT METHODS: EXPERIMENT 5

Method of calculation	Sulfate	Oxide G	Oxide H	Oxide I
Absorption mean[b]	100	94.8	90.3	91.8
Absorption (slope ratio)[c]	100	86.9	76.9	82.2
Absorption (slope ratio)[d]	100	93.0	83.0	89.0
Urinary excretion mean D 7-10[b]	100	64.6	72.5	78.6
Urinary excretion mean D 4,5[b]	100	85.3	77.6	81.8
Urinary excretion (mean - pre) D 4,5[b]	100	85.9	76.8	84.6
Urinary excretion (mean - control) Day 4,5[b]	100	73.8	59.9	67.3
Relative solubility, %				
H2O	100	<1	<1	<1
.4% HCl	100	83.9	90.0	97.3
2% Citric acid	100	79.6	68.8	96.8
Neutral ammonium citrate	100	41.3	48.3	93.9

[a]Basal practical type diet contained 1277 mg/kg Mg (as-fed basis).

[b]Based on means of groups fed 1,400 mg/kg added Mg from from all sources.

[c]Based on slope ratio calculated from 0 and 1,400 mg/kg added Mg from all sources.

[d]Based on slope ratio calculated from 0, 700, 1,400 and 2,100 mg/kg added Mg from sulfate and 0 and 1,400 mg/kg from oxides.

TABLE 30. EFFECT OF MAGNESIUM SOURCE ON DAILY URINARY MAGNESIUM EXCRETION BY SHEEP: EXPERIMENT 5[a]

	Supplemental Mg, mg/kg						
	Control	Sulfate			Oxide G	Oxide H	Oxide I
Time, d[b]	0[c]	700	1,400	2,100	1,400	1,400	1,400
Pre	348 ± 14.9[d]	312 ± 19.5	322 ± 26.4	334 ± 16.9	270 ± 33.0	255 ± 41.4	244 ± 32.1
1	332 ± 19.3	476 ± 29.4	667 ± 49.0	715 ± 66.2	567 ± 66.5[d]	506 ± 16.7	495 ± 58.5
2	374 ± 43.5	610 ± 41.4[d]	766 ± 111.4	1,024 ± 42.9	588 ± 51.1	658 ± 64.4	773 ± 110.5
3	413 ± 54.4	626 ± 56.8	877 ± 87.6[d]	1,103 ± 56.1[d]	743 ± 70.8	664 ± 68.6	612 ± 47.4
4	434 ± 35.3	812 ± 46.9	973 ± 134.3	1,282 ± 93.6	773 ± 78.9	773 ± 102.8	816 ± 79.1
5	437 ± 31.5	708 ± 63.5	1,002 ± 105.5	1,293 ± 83.3	911 ± 90.8	759 ± 106.0	798 ± 110.6
6	371 ± 12.9	622 ± 100.6	799 ± 115.7	1,003 ± 68.8	585 ± 35.7	681 ± 75.2	734 ± 142.7
7	452 ± 24.5	618 ± 90.4	861 ± 125.2	1,119 ± 63.6	647 ± 110.3	789 ± 80.4	662 ± 77.6
8	399 ± 32	690 ± 95.2	916 ± 112.7	1,149 ± 47.7	617 ± 82.3	735 ± 69.6	635 ± 91.8
9	401 ± 61.9	665 ± 104.6	776 ± 164.6	1,079 ± 55.6	613 ± 72.5	777 ± 84.1	625 ± 71.7
10	404 ± 30.0	666 ± 99.0	851 ± 162.4	1,197 ± 63.0	605 ± 80.9	717 ± 56.2	651 ± 66.4
Mean D 7-10	416	660	960	1,136	620	755	696

[a]Each value represents the mean ± standard error of five sheep.

[b]Time after feeding experimental diets. Pre represents the mean from the 2 d prior to feeding experimental diets.

[c]Control diet contained 1277 mg/kg Mg (as-fed basis).

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He is married to the former Patricia Sunny d'Hamecourt and has one daughter, Nadiuska Diane.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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